Abstracts of Invited Lectures
Poster Abstracts

Falk Symposium 165

XX International Bile Acid Meeting

BILE ACID BIOLOGY AND
THERAPEUTIC ACTIONS

Amsterdam (The Netherlands)
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President:
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P.A. Dawson, Winston-Salem  
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Session I

Metabolism and transport of bile acids
Membrane asymmetry and the regulation of bile formation

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Biological membranes have an asymmetric phospholipid composition that is maintained by flippases and floppases, mediating inward and outward phospholipid translocation, respectively. Symmetry of membranes is crucial in cell signalling, cell recognition and membrane protein function. Symmetry of the apical (canalicular) membrane of hepatocytes is particularly important for the protection against high detergent bile salt concentrations in bile. This is evidenced by two severe inherited liver diseases caused by the absence of phospholipid translocases.

Progressive Familial Intrahepatic Cholestasis (PFIC) type 3 is caused by the absence of the floppase ABCB4. This floppase mediates the outward translocation of phosphatidylcholine. This floppase function makes PC available for excretion into bile. The uptake of PC in biliary bile salt micelles reduces the detergent action of bile salt micelles and protects cell of the biliary tree.

PFIC type 1 is caused by mutations in the gene encoding ATP8B1. The role of this transporter is more complicated as it is supposed to be a flippase moving lipids in the opposite direction. The mechanism whereby impaired ATP8B1 function results in cholestasis is as yet unclear. We have extensively studied the cellular localization and the potential flippase activity of ATP8B1. We observed that ATP8B1 overexpressed in Chinese hamster ovary (CHO) cells entirely localized to the ER and not to the plasma membrane. Based on published work on homologous P-type ATPases in yeast we hypothesized that ATP8B1 requires an accessory protein from the CDC50 family for proper trafficking to the plasma membrane. The human genome bears three genes that are homologous to the yeast CDC50 gene family, and are termed CDC50A, CDC50B and CDC50C. CDC50 proteins are glycosylated ~50-kDa proteins with two putative transmembrane domains and a couple of conserved amino acid stretches. CDC50A and CDC50B are abundantly expressed whereas CDC50C is predominantly expressed in the testis. We have isolated cDNAs encoding CDC50A and CDC50B and co-expressed these proteins with ATP8B1 in CHO cell lines. This dramatically changed the localization of ATP8B1 which then, together with the CDC50 proteins, localized to the plasma membrane. Moreover, overexpression of both ATP8B1 with one of the CDC50 proteins led to a very sturdy increase in NBD-PS flipping (but not in flipping of other NBD-phospholipid analogs). We could also show that ATP8B1 translocates natural PS, because annexin V binding to the plasma membrane of these cells was decreased compared to wildtype UPS-1 cells and UPS-1 cells expressing only ATP8B1. These data demonstrate that ATP8B1 needs a CDC50 protein for proper trafficking to the plasma membrane. When the protein is present on the plasma membrane it is active as a PS flippase. We do not know yet whether CDC50 proteins are also required for this activity. The next question is how this flippase activity is essential for proper bile formation. We investigated how the absence of this flippase function relates to the chronic cholestasis that develops in patients lacking ATP8B1. In isolated mouse livers we show that Atp8b1 deficiency impairs the transport of bile salts and organic anions. In a mouse model for PFIC1, we show that phosphatidylserine (PS) is exposed on the
external leaflet of the apical membrane as demonstrated by the appearance of PS in bile. As a consequence, there is strongly enhanced cholesterol and ectoenzyme extraction by bile salts. The increase in cholesterol excretion was striking and we investigated whether this is due to increased activity of Abcg5/8. To this end we crossed the Atp8b1 mutant mouse with the Abcg8-/- mouse in which biliary cholesterol excretion is severely reduced. In spite of the absence of Abcg8 in these double transgenic mice, there was strongly increased biliary cholesterol excretion, proving that this is mediated by unspecific extraction of cholesterol rather than by translocation of cholesterol. Hence, the PS flipping mediated by ATP8B1 renders the canalicular membrane more resistant towards bile salts and thereby prevents inappropriately high cholesterol extraction. The latter is probably due to a higher relative content of sphingolipids. The loss of detergent (bile salt) resistance allows massive cholesterol extraction by bile salts. We postulate that the loss of cholesterol inactivates multiple ABC transporters and causes cholestasis. If so, the cholesterol content of the membrane turns out to represent a prime mechanism of regulation of canalicular ABC transporters.
**ABCB11 (BSEP) activity is dependent on the canicular membrane cholesterol content**

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Introduction: Progressive familial intrahepatic cholestasis type 1 is caused by mutations in ATP8B1. ATP8B1 is a flippase for phosphatidylserine (PS), however, the mechanism whereby impaired ATP8B1 function results in cholestasis is unclear. Atp8b1-deficient mice display enhanced biliary output of PS and cholesterol and decreased bile salt output. We hypothesized that PFIC1 is caused by canicular phospholipid randomization and subsequent enhanced cholesterol extraction, which impairs BSEP activity. The aim of this study was was to investigate the role of membrane cholesterol content on BSEP activity.

Methods: Atp8b1-deficient mice were infused with taurocholate (TC) and biliary bile salt and cholesterol secretion was analyzed. The effect of membrane cholesterol content on ATP-dependent TC transport was analyzed in liver plasma membranes. Cholesterol content of the membranes was modulated by incubation with cyclodextrin and/or cyclodextrin-cholesterol inclusion complex.

Results: Biliary excretion of cholesterol was 2-fold increased in Atp8b1-deficient mice compared to wild-type. In addition, biliary bile salt output was 50% reduced while hepatic TC levels were 2.5-fold higher in Atp8b1-deficient mice compared to wild-type. In mouse liver plasma membranes we demonstrate a near-linear relation between BSEP activity and liver plasma membrane cholesterol content. When 80% of membrane cholesterol was depleted, the ATP-dependent TC transport was completely abrogated. This effect was reversible as cholesterol repletion of the liver membranes completely restored TC transport.

Discussion/Conclusion: BSEP transport activity is critically dependent on the membrane cholesterol content. We hypothesize that the disproportionate extraction of cholesterol from the canicular membrane in Atp8b1-deficient mice leads to reduced Bsep activity and cholestasis.
Fic1 deficient rat hepatocytes reveal structural and functional defects in the bile canalicular membrane when exposed to hydrophobic bile acids

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Mutations in \textit{FIC1} result in PFIC1, where reduced expression of the bile acid nuclear receptor FXR (NR1H4) has also been observed. In contrast, \textit{Fic1} deficient mice do not develop cholestasis, although bile salt homeostasis and excretion are disturbed when treated with hydrophobic bile salts. To elucidate the pathogenesis of \textit{FIC1} induced cholestasis, we knocked down \textit{FIC1}/\textit{Fic1} in human cells and rat hepatocytes. In rat hepatocytes, adenoviral siRNA reduced \textit{Fic1} expression by 90\% at both mRNA and protein levels when maintained in a collagen sandwich culture system. However, no significant differences were seen in the expression of \textit{Fxr}, \textit{Bsep} and other membrane transporters at mRNA or protein levels, whereas \textit{Shp} mRNA was significantly reduced. \textit{Fxr} activity was intact in these cells as evidenced by the induced expression of its targets \textit{Shp} and \textit{Bsep}. These results were confirmed in \textit{FIC1} knockdown Caco2 and HepG2 cells. Meanwhile, \textit{Bsep} and \textit{Mrp2} were localized to the canalicular membrane in \textit{Fic1} knockdown hepatocytes, where intact canalicular membrane structures were observed from EM images. In contrast, when treated with CDCA, EM revealed that the canalicular membrane was disrupted in many \textit{Fic1} knockdown hepatocytes, but not in control cells. Further, canalicular excretion of CGamF (a fluorescent substrate of \textit{Bsep}) but not an \textit{Mrp2} substrate was significantly reduced in \textit{Fic1} knockdown hepatocytes. Therefore, we conclude that \textit{Fic1} knockdown hepatocytes maintain expression of bile salt transporters and \textit{Fxr} activity although bile salt excretion is reduced. \textit{Fic1} deficiency predisposes the canalicular membrane to injury by hydrophobic bile acids, explaining the development of PFIC1.
Bile acids and the brain. Suggested pathogenetic mechanism in connection with formation of brain xanthomas in patients with CTX

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The mechanism behind formation of cholesterol- and cholestanol-containing xanthomas in the brain of patients with a deficiency of CYP27A1 (Cerebrotendinous Xanthomatosis, CTX) has not been hitherto explained. Mice with a deficiency of Cyp27A1 do not develop xanthomas in tendons or brain and have normal or only slightly elevated levels of cholestanol in the circulation. In patients with CTX the reduced formation of chenodeoxycholic acid, the major physiological suppressor of CYP7A1 in man, leads to a marked upregulation of CYP7A1 with very high circulating levels of 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one. Because of the fact that cholic acid rather than chenodeoxycholic acid is the major suppressor of Cyp7A1 in mice, mice with defect Cyp27A1 have less increased levels of the above oxysterols. We have shown previously that a major part of the cholestanol present in the circulation of CTX patients is derived from 7α-hydroxylated intermediates in bile acid synthesis. We have now demonstrated a very efficient transfer of 7α-hydroxy-4-cholesten-3-one across cultured porcine brain endothelial cells (a model for the blood-brain barrier) that is about 100 times more efficient than the transfer of cholestanol. Furthermore, there was an efficient conversion of 7α-hydroxy-4-cholesten-3-one into cholestanol in cultured neuronal and glial cells as well as in monocyte-derived macrophages of human origin. In accordance with an impermeability of the blood-brain barrier towards cholestanol, there was no significant accumulation of cholestanol in the brain after treatment of wild type or Cyp27−/− mice with cholestanol (2%) in diet. In a pilot experiment we treated a wild type and a Cyp27−/− mouse with daily intraperitoneal injections of 7α-hydroxy-4-cholesten-3-one for a period of two months. This treatment caused intracerebral accumulation of cholestanol corresponding to about 2% and 11% of the sterol faction, respectively. In brain from CTX patients the cholestanol is present in xanthomas together with cholesterol. In vivo experiments on rats as well as in vitro experiments on human macrophages are consistent with the possibility that a primary accumulation of cholestanol may increase local cholesterol synthesis. It is suggested that the formation of cholestanol and cholesterol-containing xanthomas in the brain of patients with untreated CTX is a consequence of a flux of 7α-hydroxy-4-cholesten-3-one over the blood-brain barrier.
Session II

Transport of bile acids
Canalicular microdomains and bile formation
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Canalicular bile formation critically depends on the coordinate action of ATP-binding cassette (ABC) transporters. Multidrug resistance associated protein (MRP2 or ABCC2) is largely responsible for the generation of bile salt-independent bile flow while the bile salt export pump (BSEP or ABCB11) generates the bile salt dependent bile flow. Secreted bile salts mediate canalicular release of phosphatidylcholine, a process strictly depending on functional multidrug resistance protein 3 (MDR3 or ABCB4). Bile salts and phosphatidylcholine form mixed micelles in bile, which in turn act as acceptors for highly lipophilic compounds such as cholesterol. Canalicular excretion of cholesterol is also depending on an ABC-transporter, namely the heterodimeric ABCG5/ABCG8.

Analysis of the lipid composition of the canalicular membrane revealed that this membrane contains a large amount of sphingomyelin, which in other cell types is known to be concentrated in membrane microdomains called rafts. It is therefore conceivable that the canalicular membrane, similar to other cells, may contain microdomains. Typically, the presence of lipid rafts in a given membrane is investigated by its extraction at cold temperature with detergents such as Triton X-100 or Lubrol WX followed by isolation of detergent resistant membranes (DRMs) with sucrose gradients.

Highly purified canalicular plasma membrane vesicles were extracted at cold temperature with Triton X-100 or Lubrol WX and thereafter subjected to flotation in sucrose density gradients. DRMs could be isolated with both detergents thus indicating the presence of microdomains in the canalicular plasma membrane. Quantitative analysis of these DRMs demonstrated the complete partitioning of the raft markers alkaline phosphatase and sphingomyelin into both, Triton X-100 and Lubrol WX DRMs. In contrast, cholesterol was predominantly found in Lubrol WX DRMs only, whereas caveolin-1 was found in Triton X-100 DRMs only. Similarly, canalicular ABC transporters partitioned into Lubrol WX DRMs, but not into Triton X-100 DRMs. In conclusion, these data indicate that the rat liver canalicular membrane contains two types of microdomains, Triton rafts and Lubrol rafts. The ABC transporters involved in canalicular bile formation partition into Lubrol rafts, which are enriched in cholesterol. Hence, Lubrol rafts might be the source for biliary cholesterol.
Regulation of the cell surface expression and transport capacity of BSEP by small chemical molecules

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The efficient biliary excretion of bile acids is mediated by the bile salt export pump (BSEP/ABCB11), an ATP-binding cassette transmembrane transporter located on the bile canalicular membrane. The functional repression of BSEP induces severe cholestasis. Progressive familial intrahepatic cholestasis type 2 (PFIC2) is a lethal hereditary disease caused by a mutation in the bile salt export pump (BSEP/ABCB11) gene. We have previously demonstrated that E297G and D482G BSEP, which are frequently found mutations in European patients, result in impaired membrane trafficking, while both mutants retain their transport functions (1). Accordingly, restoration of the reduced cell surface expression of these mutated BSEPs is an important task for achieving the therapeutic goal for PFIC2 patients with E297G and D482G mutations. Since 4-phenylbutyrate (4PBA), an FDA approved drug for urea cycle disorders, has been shown to restore the reduced cell surface expression of mutated plasma membrane proteins, we investigated the effect of 4PBA treatment on E297G and D482G BSEP. Transcellular transport and cell surface biotinylation studies using Madin-Darby canine kidney II cells demonstrated that 4PBA treatment increased the functional cell surface expression of wild-type (WT), E297G, and D482G BSEP (2). The prolonged half-life of the cell-surface-resident BSEP produced by 4PBA treatment was responsible for this result (2). Moreover, treatment of Sprague-Dawley (SD) rats with 4PBA resulted in an increase in Bsep expression at the canalicular membrane, which was accompanied by an increase in the biliary excretion of [3H]taurocholic acid (2).

In conclusion, 4PBA treatment with a clinically achievable concentration induces the functional cell surface expression of WT, E297G, and D482G BSEP in MDCK II cells, and also induces functional Bsep expression at the canalicular membrane in vivo. 4PBA is a potentially useful pharmacological agent for treating not only PFIC2 patients with E297G and D482G mutations but also other cholestatic patients, in whom the BSEP expression at the canalicular membrane is reduced. However, from the in vivo study using SD rats, it appears that administration of a high dose is needed for a satisfactory effect. To improve its clinical application, further studies are underway to identify more effective agents by screening the compounds with a structural similarity to 4PBA.

References:


The role of *ABCB11* (BSEP) variation in susceptibility to intrahepatic cholestasis of pregnancy

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**Introduction:** Intrahepatic cholestasis of pregnancy (ICP) has a complex aetiology with a significant genetic component. Homozygous *ABCB11* mutations cause a spectrum of cholestatic disease. Two common European mutant alleles (E297G, D482G) have been identified, together with an ICP-specific mutation (N591S). A polymorphism (valine 444 alanine, c.1331T>C) has been reported to be associated with ICP and drug-induced cholestasis.

**Methods:** We investigated the role of E297G, D482G, N591S and V444A in ICP by screening 491 cases. A control group (n = 261) with uncomplicated pregnancies were also typed for V444A. PCR primers were used to amplify and sequence DNA using an ABI 3100 genetic analyser and results checked by an independent observer.

**Results:** E297G was identified 4 times and D482G once. In addition, N591S was identified in three cases. Allelic analysis (C vs. T) of V444A showed association of the C variant with ICP (OR 1.70, 95% CI 1.4–2.1), p < 0.0001. Genotype frequencies between the case and control groups differed significantly (Chi-squared 22.78, p < 0.001). Genotypic comparisons of homozygous individuals showed that women with CC vs. TT were more likely to develop ICP (OR 2.8, 95% CI 1.7–4.4), p < 0.0001 and CC vs. CT heterozygotes also showed increased risk (OR 1.9, 95% CI 1.3–2.6), p = 0.0003.

**Discussion/Conclusion:** *ABCB11* variability plays a significant role in the aetiology of ICP. Heterozygosity for the common mutants alleles is rare (1%), but N591S is a recurrent mutation. In the large cohort studied we have confirmed the C allele of the 444A polymorphism as a risk factor for ICP.
Intracellular transport of bile salts

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Bile salts are produced and secreted by the liver and are required for intestinal absorption of fatty food components and excretion of endo- and xenobiotics. They are reabsorbed in the terminal ileum and transported back to the liver via the portal tract. There is detailed knowledge about the dedicated bile salt transporters in hepatocytes and enterocytes that are responsible for the unidirectional transport of bile salts in the enterohepatic cycle.

**NTCP**: The sodium-dependent taurocholate-co-transporting polypeptide (NTCP, SLC10A1) is the main transporter for bile salts from the portal blood into the hepatocyte.

**BSEP**: The Bile Salt Export Pump (BSEP, ABCB11) is the hepatocanalicular bile salt transporter responsible for the concentration of bile salts into bile.

**ASBT**: Bile salts are reabsorbed at the terminal ileum by the apical sodium-dependent bile acid transporter (ASBT/SLC10A2).

**Ostα/Ostβ**: The enterohepatic circulation of bile salts is completed by the heterodimeric organic solute transporter Ostα-Ostβ (Ostαβ) that transports bile salts from the enterocyte to the blood.

However, bile salts also cross organellar membranes and there is very little knowledge about these processes and their involvement in cholestatic diseases.

Enzymes involved in bile salt biosynthesis reside in different subcellular locations, including the endoplasmic reticulum, mitochondria, cytosol and peroxisomes. Recently, we showed that the enzyme responsible for the final step in bile salt synthesis, the bile acid-CoA:amino acid N-acyltransferase (BAAT), is a typical peroxisomal enzyme in human and rat hepatocytes. BAAT is the only enzyme that catalyses the conjugation of bile salts to glycine or taurine. The peroxisomal location of BAAT has also important implications for the enterohepatic cycling of bile salts. Variable amounts of bile salts are de-conjugated by intestinal bacteria. After returning to the liver, these de-conjugated bile salts need to be re-conjugated and this requires shuttling through peroxisomes. This implies the presence of yet unknown bile salt transporters in the peroxisomal membrane. The flux of bile salts through hepatic peroxisomes is very efficient, since unconjugated bile salts fed to -or infused into- rats or mice turn up as conjugated bile salts in the bile even after a single pass through the liver. The efficiency of glycine/taurine-conjugation of bile salts is further substantiated by the fact that serum of patients treated with unconjugated ursodeoxycholic acid contains almost exclusively conjugated bile salts.

We established an in vitro assay to study the dynamics of bile salt conjugation and transcellular and transperoxisomal transport in cultured rat hepatocytes using stable isotope-labeled cholic acid. Using this assay, we provide evidence that unconjugated bile salts entering the hepatocyte shuttle through peroxisomes for taurine- or glycine-conjugation.
Conjugated bile acids regulate hepatic gluconeogenic genes via Gαi protein coupled receptor(s) in the liver

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Bile acids are synthesized from cholesterol in liver hepatocytes, secreted into bile and undergo enterohepatic circulation. In the gallbladder, they function to keep cholesterol in solution. In the intestines they are essential for the solubilization and absorption of cholesterol, lipids, and fat soluble vitamins. Recent studies show that bile acids also function as important regulatory molecules helping to control cholesterol, phospholipid, lipoprotein, bile acid and glucose metabolism in the liver. They are able to regulate the expression of various genes and the activities of specific enzymes through two distinct but yet highly coordinated cellular mechanisms. Bile acids can activate specific nuclear receptors (FXR, PXR, vitamin D) and several cell signaling pathways (ERK1/2, AKT, JNK1/2, PKC). Both free and conjugated bile acids activate the AKT (insulin signaling) pathway. Conjugated bile acids activate AKT via Gαi protein coupled receptor(s) in the liver. Activation of the AKT pathway ultimately stimulates glycogen synthase activity and glycogen synthesis. In the current study, we use primary rat hepatocytes and the chronic bile fistula (CBF) rat to study bile acid activated cell signaling, the regulation of glycogen synthase (GS) activity and gluconeogenic genes. In primary rat hepatocytes, deoxycholic acid (DCA), taurocholic acid (TCA) or insulin activated GS activity ~1.4-fold. However, the addition of insulin plus bile acids activated GS ~1.8-fold. In the CBF rat infusion of TCA for 1 h. activated AKT, GSK3 and GS by 10-fold, 4-fold, and 4-fold, respectively. In the CBF rat, TCA up-regulated SHP mRNA levels 15-fold within 3 hours. In these same animal livers, the mRNA levels of the gluconeogenic genes encoding PEP carboxykinase (PEPCK) and glucose 6-phosphatase (G-6-Pase) were markedly down regulated. In primary rat hepatocytes incubated in the absence of added insulin, the addition of TCA rapidly (0.5–4 h) down-regulated the mRNA levels of PEPCK and G-6-Pase. This down-regulation was blocked by treating hepatocytes with pertussis toxin. In primary rat hepatocytes, TCA induced SHP ~5-fold in 3 hours. Surprisingly, treatment of hepatocytes with pertussis toxin or the PI3 kinase inhibitor, wortmannin, markedly inhibited the ability of TCA to induce SHP in these cells. These data suggest that bile acids activated cell signaling pathways and nuclear receptors crosstalk to regulate glucose metabolism in the liver.
Session III

Transport and actions of bile acids
Role of the organic solute transporter OSTα-OSTβ in intestinal basolateral bile acid transport and bile acid homeostasis

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In contrast to ileal apical brush border membrane transport, the identity of the carrier(s) responsible for basolateral bile acid export into the portal circulation remained to be determined. Several candidate intestinal basolateral transporters were identified including Mrp3 (gene symbol: Abcc3) and the heteromeric organic solute transporter Ostα-Ostβ. While Ostα-Ostβ’s ileal expression, basolateral membrane localization, and bile acid transport properties were generally consistent with a role in intestinal bile acid absorption, the in vivo functions of Ostα-Ostβ had not been investigated. To determine the role of Ostα-Ostβ in intestinal bile acid transport, the Ostα gene was disrupted by homologous recombination in mice. Ostα null mice were physically indistinguishable from wild type mice with the exception of a growth deficit prior to weaning and an increased small intestinal length and intestinal hypertrophy. In everted gut sac experiments, trans-ileal transport of taurocholate was reduced 80% in female Ostα null and almost 95% in male Ostα null mice versus wild type mice; the residual taurocholate transport in female mice was in part due to Mrp3 as taurocholate transport was further reduced to near background levels in gut sacs prepared from female Ostα-Mrp3 double knockout mice. The bile acid pool size was significantly reduced (> 65%) in Ostα null mice, but paradoxically the fecal bile acid excretion was not elevated. The decreased pool size in Ostα null mice resulted from reduced hepatic Cyp7a1 expression that was inversely correlated with an elevated ileal expression of fibroblast growth factor 15 (FGF15). After acute feeding of exogenous cholic acid to overcome the reduced hepatic bile acid biosynthesis, the intestinal absorption defect was unmasked and Ostα null mice exhibited a significant increase in fecal bile acid excretion as compared to wild type mice. These data indicate that Ostα-Ostβ is essential for intestinal bile acid transport in mice. Unlike a block in intestinal apical bile acid uptake, genetic ablation of basolateral bile acid export disrupts the classical homeostatic control of hepatic bile acid biosynthesis. The combination of reduced return of bile acids in the enterohepatic circulation and decreased hepatic bile acid synthesis might be exploited therapeutically to relieve the hepatic bile acid burden in some forms of cholestatic liver disease.
Organic solute transporter (Ost) α and β are located on the basolateral membrane of enterocytes and may be responsible for the intestinal absorption of many substrates including bile salts. We have examined the mechanism governing the transcriptional regulation of their expression. In order to clarify the transcriptional regulation of Ost, reporter gene assays were performed using mouse Ost αβ promoter-luciferase reporter constructs. Co-transfection of the constructs with farnesoid X receptor (FXR) and retinoid X receptor alpha (RXRα) or liver X receptor alpha (LXRα) and RXRα into Caco-2 cells induced the transcriptional activities of both Ost α and β and further increases were observed following treatment with each agonist. Sequence analyses indicated the presence of IR-1 regions in Ost α and β promoters, which was confirmed by the finding that the deletion of IR-1 sequences abolished the response to FXR and LXRα. Furthermore, mutations in IR-1 reduced the FXR- and LXRα-dependent transactivation of Ost αβ. Together with the detection of direct binding of FXR/RXRα and LXRα/RXRα to the IR-1 elements, the presence of functional FXRE/LXRE was revealed in the promoter region of both Ost α and Ost β. In addition, the stimulatory effect of FXR/RXRα and LXRα/RXRα on Ost α, but not on Ost β, was further enhanced by HNF-4α. It was concluded that LXRα/RXRα transcriptionally regulate mouse Ost αβ via IR-1 elements shared with FXR/RXRα. Exposure to FXR/LXRα modulators may affect the disposition of Ost αβ substrates.

In addition, the fact that FXR is responsible for the transcriptional regulation of Ost αβ may be important in accounting for the pathology of PFIC 1 in the small intestine. It is reported that the expression of FXR in the nuclear is reduced in PFIC1 patients, which may result in the reduced expression of Ost αβ in the intestine. This decrease in Ost αβ in PFIC1 patients may be related to the pathogenesis of PFIC1 in the small intestine; Ost αβ substrates including bile salts may not be efficiently exported from enterocytes. From this standpoint of view, we have examined the effect of FIC1 mutants, whose sequences are reported in PFIC1 patients, on the transcriptional regulation mediated by FXR responsible element, and found the reduced function of FIC1 mutants. The results will be discussed in detail in the presentation.
Expression and localization of the membrane-bound bile acid receptor TGR5 in human gallbladder tissue

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Introduction: The G-protein coupled receptor TGR5 is the only membrane-bound bile acid receptor known to date. We have recently localized TGR5 in different non-parenchymal cells of rat liver, such as sinusoidal endothelial cells, Kupffer cells and cholangiocytes. However, localization of human TGR5 has not been studied so far, due to a lack of reliable antibodies.

Methods: TGR5 was cloned from human liver and tagged with the yellow fluorescent protein (hTGR5-YFP). An antibody against human TGR5 was generated and tested on hTGR5-YFP-transfected cells. Human gallbladder tissue was obtained by cholecystectomy. TGR5 expression and localization was analyzed by quantitative realtime PCR and immunofluorescence staining.

Results: TGR5 mRNA was abundantly expressed in all 13 gallbladders. There was no correlation of TGR5 mRNA levels with the development of gallstone disease. Immunofluorescence staining of gallbladder kryosections with the anti-TGR5 antiserum showed a strong signal in the epithelium. Antibodies against MRP2 (ABCC2) and MRP3 (ABCC3) were used to stain the apical and basolateral membranes, respectively. Triple-labelling revealed that TGR5 was localized within and in close vicinity to the apical membrane. Colocalization with rab-11 identified this "subapical" compartment as the apical recycling endosome. Incubation with tauroliothocholate increased cAMP in gallbladder tissue, indicating functional activity of TGR5.

Discussion/Conclusion: This is the first report on the localization of human TGR5. In gallbladder epithelial cells TGR5 is localized in the apical membrane, where it can be activated by tauroliothocholate. Furthermore, detection of TGR5 in apical recycling endosomes suggests, that the receptor can be regulated by rapid endocytic retrieval and exocytic insertion.
Bile salts control the antibacterial peptide cathelicidin through nuclear receptors in human biliary epithelial cells

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Introduction: Under normal conditions the biliary tract is a microbial free environment. The absence of microorganism colonization has been linked to defense mechanisms that include the physicochemical actions of bile salts. Here, we analyzed whether bile salts may also induce innate defenses in human biliary epithelial cells through nuclear receptors.

Methods: The expression of cathelicidin, a major antimicrobial peptide, was analyzed in human liver by immunostaining and real time RT-PCR. The regulation of cathelicidin expression by the therapeutic bile salt, ursodeoxycholic acid (UDCA) and the endogenous bile salt, chenodeoxycholic acid (CDCA), was assessed in human biliary epithelial cells in which native nuclear receptor expression was blunted by siRNA or dominant negative strategies.

Results: In human liver, bile ducts, which are constantly exposed to bile salts, were positive for cathelicidin immunostaining. In cultured human biliary epithelial cells, CDCA and UDCA induced cathelicidin expression. Both bile salts were able to increase vitamin D nuclear receptor (VDR) expression and activity. However, the induction of cathelicidin expression by CDCA was mediated through the farnesoid X receptor, while UDCA was active through VDR. Vitamin D potentiated cathelicidin expression stimulated by both CDCA and UDCA. In vivo, UDCA caused an increase in liver cathelicidin transcript expression in primary biliary cirrhosis patients.

Discussion/Conclusion: In conclusion, we show that bile salts may participate in biliary tract sterility through the control of epithelial cell innate immunity via nuclear receptors. Furthermore, our results suggest that in inflammatory biliary diseases associated with bacterial infection vitamin D could enhance the beneficial therapeutic effect of UDCA.
Session IV

Nuclear receptor regulation
Nuclear receptor regulation of bile acid transporters

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Uptake of bile acids across the basolateral membrane of hepatocytes is mediated mainly by the Na+-taurocholate cotransporting polypeptide NTCP (SLC10A1), but also by Na+-independent uptake transporters such as the organic anion transporting polypeptides OATP1B1 (SLCO1B1) and OATP1B3 (SLCO1B3). Several regulatory cascades have been identified that control the expression of bile acid transporters. We have previously shown that the farnesoid X receptor FXR activates transcription of the drug and peptide transporter OATP1B3 (SLCO1B3) (1), and of the heterodimeric organic solute transporters OST-α/OST-β, located at the basolateral membrane of enterocytes (2). The NTCP gene is activated transcriptionally by the glucocorticoid receptor, GR (3). A transcriptional repressor termed SHP (short heterodimer partner) negatively interferes with GR-mediated induction of NTCP gene expression. SHP expression is induced by bile acids through binding of FXR to the SHP gene. Thus bile acids can inhibit expression of NTCP by negative feed-back. A second member of the steroid receptor family, the estrogen receptor (ER), activates transcription of OATP1B1 (SLCO1B1), expressed in hepatocytes, and of the apical sodium-dependent bile acid transporter ASBT (SLC10A2), expressed in the ileum. Exogenous expression of ERα and ERβ increases the promoter activity of both the SLCO1B1 and SLC10A2 genes and the presence of the ER ligand 17-β-estradiol enhances ER-mediated activation (4). Promoter mutagenesis shows that in the context of both transporter genes, the ER response is mediated through the binding of hepatocyte nuclear factor-1α (HNF-1α). HNF-1α binding sites are located in the proximal region of the human SLCO1B1 promoter and in the 5'-UTR of the human SLC10A2 gene (5, 6). In EMSA and chromatin immunoprecipitation assays, DNA-binding of HNF-1α and recruitment of HNF-1α to the respective binding sites are increased by ERs and 17-β-estradiol (4). These results suggest a novel signaling cascade, by which ERs and estrogens activate the expression of OATP1B1 and ASBT via HNF-1α response elements. By this mechanism, estrogens may promote bile acid reabsorption in ileum by ASBT and hepatic extraction of drugs and organic anions from portal blood by OATP1B1.

References:


4. Eloranta JJ, Hiller C, Kullak-Ublick GA. Estrogen receptors activate the expression of the human enterohepatic transporter genes SLC10A2 and SLCO1B1 by enhancing the DNA-binding of HNF-1α [abstract]. Hepatology 2007; 46: 782A.


Orphan nuclear receptor LXR in bile acid detoxification and cholestasis

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We studies orphan nuclear receptor-mediated regulation of genes encoding drug metabolizing enzymes and transporters. The same enzyme and transporter systems are also responsible for the detoxification and homeostasis of numerous endogenous substances, including bile acids. As such, the nuclear receptor-mediated gene regulatory network has broad implications not only in drug metabolism, but also in establishing orphan nuclear receptors as potential therapeutic targets for many human diseases, such as the bile acid-related cholestasis. We are interested in the roles of pregnane X receptor (PXR), constitutive androstane receptor (CAR), liver X receptor (LXR) and retinoid-related orphan receptor (ROR) in bile acid detoxification. To better understand the in vivo function of these receptors, we have created a wide array of genetic engineered mice that include transgenic, knockout and “humanized” mice. Various liver disease models, such as cholestasis, are incorporated into the animal models. This presentation will focus on the novel role of LXR, by itself or in crosstalk with ROR, in regulating bile acid metabolizing enzymes and transporters, and the implications of this regulation in cholestasis.
The nuclear hormone receptor ERalpha plays a critical role in determining estrogen-induced cholesterol gallstones

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Introduction: Cholesterol gallstones are more common in women than men in every population that has been studied. This difference between women and men begins during puberty and continues through the childbearing years, focusing attention upon the effects of female sex hormones. The increased risk of gallstones in women vs. men is related to differences in how the liver metabolizes cholesterol in response to estrogen. More recently, we found that the hepatic ERalpha, but not beta, plays a crucial role in the formation of gallstones in mice challenged to high levels of estrogen. Despite these observations, the metabolic abnormalities, at a molecular level, underlying cholelithogenesis determined by ERalpha are not yet fully understood.

Methods: To explore the effect of ERalpha on gallstones, at weaning, female ERalpha (-/-) and (+/+) mice were ovariectomized. At 8 weeks of age, the mice were implanted subcutaneously with pellets releasing 17beta-estradiol (E2) at 6 microgram/day and fed a lithogenic diet for 8 weeks. Cholesterol crystallization and gallstones in gallbladder bile were quantified by microscopy. To monitor changes in hepatic cholesterol biosynthesis and the newly synthesized cholesterol secreted into bile in ovariectomized mice in response to E2 and its antagonist ICI 182,780, mice were injected intravenously with 2.5 mCi of [3H]water, as well as the incorporation into digitonin-precipitable sterols and fatty acids was measured in liver extracts 1 hour later and hepatic bile was harvested every hour for 8 hours, respectively. To investigate whether ERalpha activated by estrogen impairs signal transduction by inhibiting CCK-1 receptor (CCK-1R)-G protein coupling on gallbladder smooth muscle, E2-treated mice were administrated intraperitoneally with the CCK-1R antagonist devazepide at 0 or 1 mg/day. Gallbladder contraction in response to exogenous CCK-8 was determined. Gene expression and protein levels of the hepatic cholesterol biosynthetic pathway, CCK-1R, ERalpha, and G-proteins were measured by real-time PCR and Western methods.

Results: Cholesterol crystallization and gallstone formation were greatly accelerated in (+/+) mice in challenge to high levels of E2, mainly through up-regulating hepatic expression of ERalpha. The lithogenic actions of estrogen can be abolished by deletion of the ERalpha gene. Compared with (-/-) mice, E2-treated (+/+) mice displayed significantly increased expression levels of mRNAs for SREBP-2 and four major genes of cholesterol biosynthesis pathway, whatever chow or lithogenic diet is fed. Furthermore, E2 profoundly stimulates sterol synthesis as reflected in increased hepatic enzymatic activity of HMG-CoA reductase. A massive increase in sterol synthesis (8-fold) and a lesser increase in fatty acid synthesis (twofold) were found in (+/+) mice, but not in (-/-) mice. The increase in hepatic cholesterol synthesis is associated with a significant increase in biliary secretion rates of total and newly synthesized cholesterol. However, the E2 effects on increasing cholesterol
biosynthesis are blocked by deletion of the ERalpha gene and by ICI 182,780. The marked reduction in cholesterol synthesis correlates with the significant decrease in the amount of mRNAs for SREBP-2 and multiple genes of cholesterol biosynthesis and in biliary secretion rates of total and newly synthesized cholesterol in (-/-) mice and in (+/+) mice treated with E2 plus ICI 182,780. Fasting gallbladder volumes were significantly larger in (+/+) mice than in (-/-) mice in exposure to high doses of E2. In the gallbladders of E2-treated (+/+) mice, expression levels of ERalpha were up-regulated and expression levels of CCK-1R and CCK binding capacity of gallbladder CCK-1R’s were reduced. Thus, CCK-8-mediated gallbladder emptying was diminished and resulted in enlarged fasting and residual gallbladder volumes, mostly contributable to impaired gallbladder contractility. In contrast, these gallbladder phenotypes were not detected in (-/-) mice exposed to E2, in which fasting and residual gallbladder volumes remained normal. Whereas, protein concentrations of Galpha-i3 were significantly reduced and Galpha-s content was increased in the gallbladders of (+/+) mice at week 8 compared with those on day 0, this was not the case in (-/-) mice. When the gallbladder CCK-1R was blocked by its antagonist devazepide, (+/+) mice showed the E2 modulation of G protein function with an increase in protein concentrations of Galpha-s but not in Galpha-i3. In contrast, these changes were not observed in (-/-) mice.

**Discussion/Conclusion:** (1) ERalpha plays an important role in the regulation of cholesterol metabolism by stimulating SREBP-2 that activates SREBP-2-responsive genes in the cholesterol biosynthetic pathway. (2) During the E2 treatment, mice continue to synthesize cholesterol in spite of its excess availability from the lithogenic diet, which reflects a loss in controlling the negative feedback regulation of cholesterol biosynthesis so that mice fail to down-regulate hepatic cholesterol biosynthesis and more newly-synthesized cholesterol could be secreted into bile leading to biliary cholesterol hypersecretion. (3) The E2-ERalpha pathway regulates gallbladder motility through both CCK-1R and G proteins in the gallbladder. (4) Overexpression of ERalpha stimulated by E2 induces signal-transduction decoupling in the gallbladder smooth muscle and decreases gallbladder contractile in response to CCK, which results in gallbladder stasis and enhanced cholelithogenesis.
Role of intestinal nuclear bile acid receptor FXR in the gut-liver axis

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Bile acids are endogenous molecules synthesized in the liver as end product of cholesterol metabolism. Secreted in bile, stored in the gallbladder in the inter-prandial period, bile acids are released in the intestine in response to food intake. After active up-take in the ileum, bile acids return back to the liver through the portal vein, thus completing their enterohepatic circulation. Classical physiologic functions of bile acid include promotion of hepatic homeostasis and bile flow, support of the smooth progression of lipid digestion and absorption, absorption of lipophilic vitamins, cholesterol solubilization and disposal. Moreover, bile acids are directly involved in preserving the physiologic commensal host-intestinal bacteria relationship and in the regulation of the intestinal mucosal turnover. Farnesoid X Receptor (FXR) is the nuclear receptor bile acid sensor responsible for a coherent transcriptional regulation of a series of genes that codify for proteins directly involved in the maintenance of lipid homeostasis in the gut-liver axis. It is reasonable to elect FXR as the transcriptional link for the well-known bile acid actions. Here we will present and discuss the actual role of FXR in the intestine. While novel discoveries have underscored the direct involvement of this nuclear receptor in bile acid-related pathophysiological relevant conditions, several questions should be still answered before connecting any bile acid activities to FXR.
Session V

Bile acids as metabolic integrators
LRH-1 and control of bile acid homeostasis

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Liver receptor homolog 1 (LRH-1, NR5A2) is an orphan nuclear receptor previously shown to be part of a nuclear receptor network governing bile acid homeostasis. While the role of most of the other nuclear receptors of this regulatory cascade has been underscored in studies employing germine mutant mouse models, the in vivo role of LRH-1 has remained unclear as germline LRH-1 deficient mice are embryonic lethal. In the study that will be presented, targeted somatic mutagenesis of LRH-1 in mouse hepatocytes has been used to characterize the exact contribution of this nuclear receptor in bile homeostasis and bile-mediated intestinal lipid absorption. The characterization of these hepatocyte-specific LRH-1 deficient mice unequivocally demonstrates that LRH-1 controls the expression of several enzymes in bile acid production, with CYP8B1 expression being most affected. We demonstrated that the absence of LRH-1 and subsequent deficiency of CYP8B1 results in a almost complete elimination of cholic acid (CA) and its amino acid conjugate taurocholic acid (TCA) and increases the relative amounts of less amphipathic bile acid species. The drastic remodeling of the bile acid composition in turn was shown to reduce significantly the efficacy of intestinal absorption of lipids and re-uptake of bile acids and increased the removal of lipids from the body. Our data hence underscore the importance of the commanding role of hepatic LRH-1 on the determination of bile acid composition and amphipathicity, in the control of whole body lipid homeostasis.
Differential effects of ursodeoxycholic acid (UDCA) and side-chain-shortened norUDCA in the treatment of fatty liver and atherosclerosis

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Background: Beside their well established role in the regulation of dietary lipid absorption and cholesterol homeostasis, bile acids may also play a key role as endocrine signaling molecules that coordinate hepatic lipid homeostasis through nuclear hormone receptors and thus might represent a promising therapeutic treatment strategy for non-alcoholic fatty liver disease (NAFLD) and arteriosclerosis. However, ursodeoxycholic acid (UDCA), did not improve non-alcoholic steatohepatitis (NASH) in humans (Lindor et al. Hepatology 2004) and more effective therapies are needed. Moreover, UDCA has never has been tested in models of atherosclerosis despite being known to reduce LDL-cholesterol (LDL-C). We therefore aimed to explore the potential therapeutic mechanisms of UDCA and its side chain-shortened homologue norUDCA on NAFLD and arteriosclerosis in Western-diet fed ApoE⁻/⁻ mice.

Methods: ApoE⁻/⁻ mice were fed Western diet for 8 weeks (Co). Treatment groups received either 0.5% UDCA or norUDCA in addition to Western diet from week 4–8 (i.e. after development of hepatic steatosis and atherosclerosis). H&E and red oil staining, hepatic TG-levels, expression of key genes in hepatic TG homeostasis, neutrophil infiltration and VCAM-1 expression, the degree of aortic (valve) plaque formation, and serum lipid composition were compared.

Results: norUDCA significantly reduced hepatic triglyceride content, induced FA oxidation (AOX mRNA expression), reduced TG synthesis (Lpin1 mRNA expression), neutrophil count, and VCAM expression. In addition, norUDCA significantly reduced aortic plaques surface area and aortic staining for macrophage marker F4/80. Interestingly, while UDCA treatment significantly reduced total serum cholesterol and triglyceride levels in ApoE⁻/⁻ mice, norUDCA had no effect on both parameters. However, FPLC-analysis clearly demonstrated an increase of cholesterol and phospholipids in the HDL-fraction in norUDCA treated animals when compared to Co. Moreover, norUDCA but not UDCA restored Cyp7a1 expression in Western-diet fed animals.

Conclusions: norUDCA is superior to UDCA in the treatment of NAFLD and arteriosclerosis in Western chow-fed ApoE⁻/⁻ mice and these effects are independent of total serum cholesterol and triglyceride levels. Due to its multiple effects on lipoprotein composition, foam cell formation, and hepatic lipid metabolism side-chain homologues of UDCA may represent promising drugs to treat NAFLD and arteriosclerosis.
Side-chain-modification critically determines the physiologic and therapeutic properties of 24-nor-ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knock-out mice and isolated bile duct units

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Background and aim: Nor-ursodeoxycholic acid (norUDCA) reverses sclerosing cholangitis and biliary fibrosis in multidrug resistance gene 2 knock-out (Mdr2-/-) mice. This may be critically related to the relative conjugation resistance of norUDCA undergoing cholehepatic shunting and inducing bicarbonate-rich hypercholeresis. To test this hypothesis we compared the effects of norUDCA, its taurine conjugate T-norUDCA, and further side chain shortened bis-norUDCA in Mdr2-/- mice and isolated mouse bile duct units (IBDU).

Material and methods: 8 weeks-old Mdr2-/- mice were fed a standard chow or a diet containing norUDCA, T-norUDCA or bis-norUDCA for 4 weeks. Liver histology, serum liver enzymes, bile flow, markers of liver fibrosis as well as mRNA expression of key detoxification and transport systems were compared. Potential choleretic mechanisms in cholangiocytes were addressed in IBDU in bicarbonate containing and free medium.

Results: NorUDCA but not T-norUDCA, significantly reduced ALT/AP levels and improved liver histology. In contrast, bis-norUDCA even deteriorated the cholestatic phenotype. NorUDCA and bis-norUDCA stimulated the expression of basolateral bile acid efflux pump Mrp4. Induction of bile acid biotransformation enzymes (Cyp2b10 and Sul2a1) was observed after norUDCA and bis-norUDCA treatment. Biliary bicarbonate-output was 2-fold higher in norUDCA compared to T-norUDCA-treated animals. NorUDCA stimulated bile secretion in IBDU more potently than tauro-norUDCA, effects which were partially bicarbonate-dependent.

Summary and conclusion: NorUDCA is superior to its homologues in the reduction of liver injury, ductular proliferation and periductal fibrosis in Mdr2-/- mice, suggesting bicarbonate rich hypercholeresis and cholehepatic shunting as its key mechanisms of action.
Role of side chain amidation for the anticholestatic action of norUDCA in rat liver

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Background and aim: The impact of taurine conjugation for the anticholestatic effect of ursodeoxycholic acid (UDCA) and its taurine conjugate (TUDCA) is unclear (Nature CP Gastr Hepat 2006; 3). Norursodeoxycholic acid (norUDCA) exerts therapeutic effects superior to UDCA in Mdr2/Abcb4 knockout mice (Mdr2-/-) that develop chronic progressive sclerosing cholangitis (Gastroenterology 2006; 130: 465). In contrast to UDCA, norUDCA is poorly conjugated by rat and human hepatocytes (Hepatology 2005; 42: 1319). Tauroliothocholic acid (TLCA)-induced cholestasis represents a well-established experimental model of hepatocellular cholestasis. The aim of the present study was to compare the effect of norUDCA and its taurine conjugate (TnorUDCA) on bile formation and liver cell injury in TLCA-induced cholestasis of perfused rat livers.

Methods: The effect of norUDCA and TnorUDCA (25 μmol/l, each) on bile flow and biliary secretion of the Mrp2 (Abcc2) substrate, 1,2-dinitrophenyl-S-glutathione (GS-DNP), was studied in presence or absence of TLCA (10 μmol/l) in isolated perfused rat livers (JBC 2003; 278: 17810). Bile acid administration was started after 45 min, and 1-chloro-2,4-dinitrobenzene (CDNB, 30 μmol/l), the precursor of GS-DNP, was administered from min 65 to 75. Bile secretion was determined gravimetrically, GS-DNP secretion fluorometrically, biliary bile salt composition by tandem mass spectrometry, and liver cell apoptosis by an immunohistochemical approach (caspase 3, cytokeratin 18 staining). Statistics: ANOVA with Tukey’s post-hoc test.

Results: TnorUDCA and norUDCA stimulated bile flow in control livers, but did not affect GS-DNP secretion. TnorUDCA, but not norUDCA, reversed TLCA-induced impairment of bile flow and, in part, GS-DNP secretion. TnorUDCA and norUDCA did not significantly affect TLCA-induced apoptosis. TnorUDCA was detected at millimolar levels in bile of livers treated with TLCA + TnorUDCA for 20 min, but not at relevant levels (< 0.1 mmol/l) in those treated with norUDCA.

Conclusions: Taurine conjugation is essential for the anticholestatic action of norUDCA in cholestasis induced by TLCA in perfused rat liver.
OSTα-OSTβ as a potential therapeutic target for altering bile acid and lipid levels

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The enterohepatic circulation of bile acids is essential for cholesterol homeostasis, intestinal absorption of dietary fats and vitamins, and for proper regulation of bile flow and biliary lipid secretion. A key step in this process is the intestinal absorption of bile acids, which is mediated largely by the apical sodium-bile acid uptake cotransporter Asbt/Slc10a2, and possibly by the recently described basolateral Ostα-Ostβ efflux transporter. Ostα-Ostβ was initially identified in the liver of the little skate, Leucoraja erinacea, using an expression cloning strategy, and the human and mouse orthologues were subsequently cloned and characterized (Wang et al. Proc Natl Acad Sci 98:9431–6, 2001; Seward et al. J Biol Chem 278:27473–82, 2003). This transporter is composed of a predicted seven-transmembrane-domain protein, Ostα, and a single transmembrane-domain polypeptide, Ostβ. Heterodimerization of Ostα and Ostβ is required for delivery of the functional transport complex to the plasma membrane (Li et al. Biochem J 407:363–72, 2007). Several lines of evidence support the hypothesis that Ostα-Ostβ is the key basolateral exporter of bile acids and related molecules: (a) Ostα-Ostβ mediates the transport of bile acids and conjugated steroids, including taurocholate, estrone-3 sulfate, and dihydroepiandrosterone sulfate; (b) Transport occurs by a facilitated diffusion mechanism, and thus Ostα-Ostβ can mediate either efflux or uptake depending on the electrochemical gradient of a given substrate; (c) Ostα and Ostβ expression in various tissues and along the gastrointestinal tract parallels that of Asbt; (d) Both Ostα and Ostβ proteins are localized to the basolateral plasma membrane of cells that are known to export bile acids, including enterocytes, cholangiocytes, and renal proximal tubular cells; and (e) Expression of both Ost genes is positively regulated by bile acids through the bile acid-activated farnesoid X receptor, FXR, consistent with a role of this transporter in bile acid export.

To more directly test this hypothesis, Ostα-deficient mouse models have recently been developed both in our laboratory (Li et al. Biochem J 407:363-72, 2007; and unpublished observations), and by Rao et al. (Proc Natl Acad Sci 105:3891–6, 2008). Ostα−/− mice are viable and fertile, but exhibit small intestinal hypertrophy and growth retardation. As demonstrated by Li et al. (2007), Ostα−/− mice lack both Ostα and Ostβ proteins because the individual subunits of this heteromeric transport complex are not stable in the absence of their obligate heterodimerization partner. Of significance, bile acid pool size and serum levels are decreased by more than 60% in Ostα−/− mice, whereas fecal bile acid excretion is unchanged, indicating a defect in intestinal bile acid absorption. In support of this hypothesis, when [3H]taurocholic acid or [3H]estron 3-sulfate are administered directly into the ileal lumen, absorption is lower in Ostα−/− mice. Interestingly, serum cholesterol and triglyceride levels are also ~15% lower in Ostα−/− mice, an effect that may be related to the impaired intestinal
bile acid absorption. Loss of Ostα is associated with compensatory changes in the expression of several genes involved in bile acid homeostasis, including an increase in the multidrug resistance-associated protein 3, Mrp3/Abcc3, an alternate basolateral bile acid export pump, and a decrease in cholesterol 7α-hydroxylase, Cyp7a1, the rate-limiting enzyme in bile acid synthesis. The latter finding may be explained by increased ileal expression of fibroblast growth factor 15 (Fgf15), a negative regulator of hepatic Cyp7a1 transcription.

Given this central role of Ostα-Ostβ in the absorption of bile acids and structurally related molecules, our recent studies have focused on defining the mechanisms that regulate Ostα-Ostβ functional activity. Of significance, we have now identified the specific amino acids in the Ostβ polypeptide that are necessary for binding to Ostα, and have proposed a pharmacological approach that may be used to prevent Ostα-Ostβ heterodimerization (unpublished observations). Because heterodimerization is required for Ostα and Ostβ protein stability and for delivery of the functional transporter to the plasma membrane, these findings suggest a novel approach for inhibiting intestinal bile acid absorption, and thus for altering bile acid and lipid homeostasis.
Session VI

Bile acids: Cellular injury and regeneration
Acrobatic liver stem cells: Role in cellular injury and regeneration

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Hepatic progenitor cells are immature epithelial cells that reside in the smallest ramifications of the biliary tree in human liver. These cells are capable of differentiating towards the biliary and the hepatocytic lineage and represent the human counterpart of the oval cells in rodent liver. An increased number of progenitor cells (referred to as ‘activation’) and differentiation of the same towards hepatocytes and/or bile duct epithelial cells is a component of virtually all human liver diseases. The extent of progenitor cell activation and the direction of differentiation are correlated respectively with the severity of the disease and the type of mature epithelial cell type (hepatocyte or bile duct epithelial cell) that is damaged. Analogous to findings in animal models of hepatocarcinogenesis, human hepatic progenitor cells most likely can give rise to hepatocellular carcinoma.

The factors that govern human hepatic progenitor cell activation and differentiation are beginning to be indentified. Growth factors like hepatocyte growth factor, transforming growth factor alpha and beta play a role. Recently it has been shown that also the autonomic nervous system plays a role in liver regeneration. Since the liver is denervated after liver transplantation this is of relevance for the regenerative capacity of the transplanted liver. The microenvironment is essential for the differentiation of progenitor cells. Interaction of progenitor cells with the surrounding stroma has to be further explored; the stroma contains mesenchymal cells like stellate cells/myofibroblasts, matrix components, growth factors... Using microdissection and microarray techniques, we could identify different stromal factors in biliary diseases, compared to hepatitis with parenchymal damage, in which progenitor cells differentiate into hepatocytes.

Animal data indicate that oval cells are activated when oxidative stress inhibits the regenerative capacity of more mature hepatocytes. Inhibition of replicative activity of mature hepatocytes is also shown in human liver diseases like alcoholic hepatitis and viral hepatitis. Recently, Wiemann et al. illustrated that hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis induced by a wide variety of etiologies. A study of Falkowski et al. very nicely confirms inhibition of hepatocyte replication in the cirrhotic stage of chronic viral hepatitis and that this is accompanied by an increased replication rate of the progenitor cell compartment. This increase in progenitor cell activation has also been shown in (cirrhotic) alcoholic and non-alcoholic fatty liver disease, conditions in which replication of hepatocytes is also inhibited. Overall, oxidant-induced replicative senescence, as well as the progenitor cell response, seems to be stereotypical, irrespective of the type of underlying liver disease. Progenitor cell activation is particularly pronounced in the cirrhotic stage of a variety of chronic liver diseases, the stage in which most carcinomas arise. This should be kept in mind when progenitor cells are considered as a therapeutical option in chronic liver diseases.
Several recent detailed immunohistochemical studies have shown that hepatocellular carcinomas show a range of hepatocellular, but also progenitor cell features, suggesting that at least part of the HCCs originate from progenitor cells. HCCs with progenitor/biliary features have a faster and higher recurrence rate after surgical treatment. Especially the independent prognostic value of Keratin19 in primary liver carcinoma has been extensively documented. Its validity was proven in several studies by diverse methodologies, in different centres and in different ethnic patient groups. Since the current classification (hepatocellular carcinoma, cholangiocellular carcinoma, mixed hepatocellular-cholangiocellular carcinoma, intermediate cell tumors, cholangiolocellular carcinoma, collision tumors, …) is confusing and of little prognostic value, an international study group proposes a new classification of primary liver carcinomas based on the main cell type present in the tumour and the expression and percentage of expression of CK19 in the liver cancer.
Pro- and antiapoptotic actions of bile acids and CD95 ligand in hepatic stellate cells

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Hydrophobic bile acids and CD95 ligand (CD95L) are potent inducers of apoptosis in hepatocytes, but not in quiescent hepatic stellate cells, despite expression of the CD95 death receptor (1, 2). Thus, the function of the CD95-system in quiescent HSC is unclear. The effects of CD95L and of the proapoptotic bile acid tauroliothocholysulfate (TLCS) on quiescent, 1–2 days-cultured rat HSC were studied with regard to CD95-activation, signal transduction, proliferation and apoptosis. In quiescent HSC, CD95L led to a rapid phosphorylation of the epidermal growth factor-receptor (EGFR), extracellular signal regulated kinases (Erk) and of c-Src, but not of Yes, c-Jun-N-terminal kinases and p47phox, an activating subunit of NADPH-oxidase. CD95L-induced EGFR- and Erk-phosphorylation were abolished after proteinase-inhibition by GM6001 and in presence of neutralizing EGF-antibodies, suggestive for a ligand-dependent EGFR-phosphorylation in response to CD95L. Interestingly, CD95L did not induce apoptotic cell death but stimulated HSC proliferation and simultaneously triggered a rapid inactivating CD95-tyrosine-nitration, which was no more detected in activated, 10–14 days-cultured HSC (3). EGFR-phosphorylation, HSC-proliferation and CD95-tyrosine-nitration were also triggered by tumor necrosis factor-α and TRAIL.

Also TLCS induced in quiescent HSC a rapid activation of the EGFR, which, however, was ligand-independent and due to Yes activation. TLCS induced a rapid Ser-phosphorylation of p47phox, an activating subunit of NADPH oxidase. Thus, TLCS produced in contrast to CD95L an oxidative stress signal, as observed in hepatocytes (4). No CD95-Tyr-nitration occurred in response to TLCS and the activated EGFR did not associate with CD95. However, in presence of cycloheximide (CHX), TLCS-induced EGFR activation resulted in EGFR/CD95 association, subsequent CD95-Tyr-phosphorylation and formation of the death-inducing signaling complex.

In summary, CD95L and other death receptor-ligands as well as TLCS are mitogens in quiescent HSC through EGFR-phosphorylation. CD95L-induced EGFR activation is ligand-dependent, whereas TLCS-induced EGFR activation is ligand-independent and mediated by Yes. CD95L, but not TLCS triggers simultaneously an antiapoptotic signaling by CD95L-induced CD95-tyrosine-nitration. CHX switches TLCS-induced proliferation to apoptosis by allowing for a JNK-dependent EGFR/CD95 association as prerequisite of CD95-Tyr-phosphorylation. The unusual response of quiescent HSC towards death receptor-ligands and toxic bile acids may help quiescent HSC to participate in liver regeneration following liver-injury (5).

References:


Successful reactivation of hepatic Mrp transporters in endotoxin induced cholestasis in mice by the CAR activator 6,7-dimethylesculetin

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Introduction: Inflammatory cholestasis has been previously characterized as a result of reduced organic anion transporter expression mediated by a decreased expression of class II nuclear hormone receptors (NHR). Specific ligands of NHR including CAR have been described as activators of transporter target genes. We hypothesized that treatment with the CAR activator 6,7-dimethylesculetin (DE) in endotoxemic mice may induce expression and putatively a nuclear redistribution of NHR leading to a maintained transporter gene expression.

Methods: Male C57/BL6 mice were i.p. injected with LPS (0.5 µg/kg) and simultaneously treated with 6,7-dimethylesculetin (100 mg/kg) or vehicle only (corn oil) for 4 to 16 h. Expression of NHR (CAR, PXR, FXR, RAR, RXR, Lrh-1) as well as basolateral (Ntcp, Mrp3, Mrp4) and canalicular (Mrp2, Bsep) transporters were determined by Northern Blotting or quantitative RT-PCR. Subcellular localization of CAR protein with and without DE-treatment was visualized by immunohistochemistry staining.

Results: Treatment with the CAR activator 6,7-DE in endotoxemia even induced NHR gene expression with maximum effects on CAR (155 ± 32%) and Lrh-1 (186 ± 23%) at 16 hours compared to an extensive suppression in control mice. Immunohistochemistry staining provided no DE-specific effect, since sham controls were also characterized by a partial nuclear redistribution of the receptor protein. At 16h transcriptional CAR and Lrh-1 activation was associated with a prominent induction of Mrp3 (186 ± 41%) and a maintained expression of Mrp2 compared to a -70% decrease in controls, respectively. Analysis of other transporters revealed a partial but significant recovery of Ntcp transcript numbers by DE-treatment whereas Mrp4 and Bsep remained unaffected.

Discussion/Conclusion: These results provide for the first time a novel protective interventional approach to selectively modify an otherwise unselective negative hepatic acute phase response during endotoxemia at the molecular level.
Neuropeptides, neuroendocrine hormones and bile acid interactions in cholangiocyte response to injury

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Cholangiocytes, the epithelial cells that line the intrahepatic biliary tree, are the target of cholangiopathies, a wide array of chronic disorders that are characterized by the progressive vanishing of bile ducts, leading to ductopenia and liver failure. The loss of bile ducts is a consequence of cholangiocyte death by apoptosis and impaired proliferative response of these cells to injury. The factors that regulate cholangiocyte proliferation and survival are poorly understood. In this regard, a major role is played by the interaction between bile acids and the autonomic nervous system. It has been shown that adrenergic and cholinergic denervation of the liver results in the induction of cell death and impaired proliferative responses of the biliary epithelium to cholestasis. In addition, bile acids have been shown to enter cholangiocytes through the apical, Na+-dependent bile acid transporter, ASBT, which has a marked impact on cholangiocyte pathobiology. Recent evidence shows that bile acids and autonomic innervations interact in modulating cholangiocyte response to liver injury.
Session VII

Bile acid-related genetic liver disease
The many faces of MDR3 deficiency

The relevance of canalicular membrane transporting proteins for human liver disease

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Canalicular membrane transporting proteins actively remove solutes from the hepatocytes. Their dysfunction may lead to liver disease. BSEP, or bile acid export pump (ABCB11), transports monovalent bile acids from hepatocyte to bile. Mutations of the ABCB11 gene cause deficiency of BSEP function and a severe cholestatic liver disease called progressive familial intrahepatic cholestasis type 2 (PFIC type 2). Children with PFIC type 2 are at risk to develop cholangio-hepatocellular carcinomas at a very young age. Point mutations that only partially affect BSEP function are associated with a less severe cholestatic disorder called benign recurrent intrahepatic cholestasis type 2 (BRIC type 2). Heterozygosity for BSEP mutations has been described in intrahepatic cholestasis of pregnancy (ICP) and drug-induced cholestasis but whether these mutations are causative or merely represent associations, is currently not known.

ATP8B1 is a phospholipid translocator that flips phosphatidylserine from the outer leaflet to the inner leaflet of the plasma membrane. It is highly expressed in liver, predominantly in bile ductules, in intestine and in pancreas. Mutations of the gene encoding this translocator, the FIC1 gene, are associated with PFIC type 1, which like PFIC type 2 is a severe form of cholestatic liver disease. Also here less severe mutations (point mutations affecting one amino acid in a structural domain of the protein) cause BRIC type 1, an episodic form of cholestasis that comes and goes with a tendency to become less severe later in life. Although ICP has been reported in families with PFIC type 1 children, the association between FIC1 mutations and ICP is not clear.

ABCB4/MDR3 is a transporter for phosphatidylcholine in the canalicular membrane of hepatocytes. While BSEP and ATP8B1 are associated with cholestatic human liver disease of rather limited phenotype, ABCB4 mutations are associated with a heterogeneous group of human liver diseases varying from PFIC type 3 to low phospholipid-associated cholelithiasis (LPAC), drug-induced cholestasis and ICP.

ABCB4/MDR3 in mice is called Mdr2. Mdr2 deficiency in mice causes a chronic liver disease characterized by portal inflammation, fibrosis and cholangio-hepatocellular carcinoma. The background to this disease most probably is the phospholipid-poor bile that these animals produce because of the absence of the mdr2 protein. This protein is a member of the ABC-class of transporter proteins in the canalicular membrane of hepatocytes that acts as a transporter of phosphatidylcholine (PC) flipping PC from the inner to outer membrane leaflet of the hepatocyte canalicular membrane. Via interaction with bile acids, PC is subsequently secreted into bile.
Bile without phospholipids is toxic to surrounding structures such as hepatocytes and the smaller intrahepatic bile ducts. Thus, phospholipid-poor bile in mdr2−/− mice is cytotoxic because the detergent action of bile acids is not neutralized by phosphatidylcholine that in normal bile is abundantly present. Treatment with ursodeoxycholic acid improves the liver disease in mdr2−/− mice but feeding a cholate-containing diet aggravates the disease.

Apart from PFIC type 3, a clear and well-defined human counterpart of this mouse liver disease has not been established unequivocally. This would require a disease in which phospholipid-poor bile is associated with portal inflammation and fibrosis. Such a disease has not been identified yet. In most human liver diseases associated with ABCB4 mutations, phospholipid concentrations in bile have not been determined or have been normal. Thus even for the so-called "established diseases" in the table below, the pathophysiology needs to be established.

We have searched for MDR3 mutations in human liver diseases of non-viral, non-alcoholic origin. We have found a number of interesting phenotypes some of which have not been associated before with ABCB4 gene defects. ABCB4 gene defects come in many forms: mutations can give rise to truncated proteins, no proteins or slightly abnormal proteins.

Dysfunction of MRP2 causes the conjugated hyperbilirubinemia that characterizes Dubin Johnson syndrome. Defects of any of the other canalicular membrane transporters such as MDR1 (ABCB1), ABCG5/G8, BCRP (ABCG2) have not been linked to any human liver disease yet. Apart from a one-to-one relation between a transporter defect and liver disease, one has to realize that a transporter defect, or the failure to regulate canalicular transporter expression or function under conditions of increased demand, could modify the course or the expression of a liver disease. Therefore, as modifier genes any of the transporters could play a role in polygenic diseases such as primary biliary cirrhosis, primary sclerosing cholangitis and drug-induced cholestasis. In this regards it is of interest that a number of the ATP-dependent transporters (ABCG2/BCRP, ABCC3/MRP3, ABCB1/MDR1) are highly expressed in hepatic progenitor cells and thus could play a role in liver regeneration. Dysfunction of these transporters could be the cause of early apoptosis of progenitor cells and the inability to respond properly to a toxic stimulus.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Clinical phenotype</th>
</tr>
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| FIC 1 | ATP8B1 | Established relation:
|  |  | Progressive familial intrahepatic cholestasis type 1 |
|  |  | Benign recurrent intrahepatic cholestasis type 1 |
|  |  | Putative relation:
|  |  | Intrahepatic cholestasis of pregnancy |
| ABCB11 | BSEP | Established relation:
|  |  | Progressive familial intrahepatic cholestasis type 2 |
|  |  | Cholangio-hepatocellular carcinoma |
|  |  | Benign recurrent intrahepatic cholestasis type 2 |
|  |  | Putative relation:
|  |  | Intrahepatic cholestasis of pregnancy |
|  |  | Drug-induced cholestasis |
| ABCB4 | MDR3 | Established relation:
|  |  | Progressive familial intrahepatic cholestasis type 3 |
|  |  | Low phospholipid-associated cholelithiasis |
|  |  | Intrahepatic cholestasis of pregnancy |
|  |  | Putative relation:
|  |  | Biliary cirrhosis* |
|  |  | Idiopathic adulthood ductopenia* |
|  |  | Drug-induced cholestasis |
|  |  | Biliary pancreatitis |
|  |  | HELPP syndrome* |
|  |  | Post-transplantation biliary strictures |
|  |  | Oriental hepatolithiasis |

* will be discussed during Falk meeting
Severe bile salt export pump deficiency: Mutations, immunohistochemistry and malignancy risk

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Introduction: Patients with severe bile salt export pump (BSEP) deficiency present as infants with progressive cholestatic liver disease. We characterised mutations of ABCB11 (encoding BSEP) in such patients and correlated genotypes with residual protein detection and malignancy risk.

Methods: Patients with intrahepatic cholestasis suggestive of BSEP deficiency were investigated by single-strand conformational polymorphism analysis and sequencing of ABCB11. Genotypes sorted by likely phenotypic severity were correlated with data on BSEP immunohistochemistry and clinical outcome.

Results: Eighty-two different mutations (52 novel) were identified in 109 families (9 nonsense mutations, 10 small insertions and deletions, 15 splice-site changes, 3 whole-gene deletions, 45 missense changes). In 7 families only a single heterozygous mutation was identified despite complete sequence analysis. Thirty-two percent of mutations occurred in > 1 family, with E297G and/or D482G present in 58% (52/89) of European families. On immunohistochemical analysis (88 patients), 93% had abnormal or absent BSEP marking. Expression varied most for E297G and D482G, with some BSEP detected in 45% (19/42) of patients with these mutations.
Hepatocellular carcinoma or cholangiocarcinoma developed in 15% (19/128) of patients. Two protein-truncating mutations conferred particular risk; 38% (8/21) of such patients developed malignancy versus 10% (11/107) with potentially less severe genotypes (relative risk 3.7 [CL = 1.7–8.1, p = 0.003]).

**Discussion/Conclusion:** With this study, > 100 ABCB11 mutations are now identified. Immunohistochemically detectable BSEP is typically absent, or much reduced, in severe disease. BSEP deficiency confers risk of hepatobiliary malignancy. Close surveillance of BSEP-deficient patients retaining their native liver, particularly those carrying 2 null mutations, is essential.
Role of genetics in drug-induced cholestasis

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Drug-induced liver injury is an important clinical problem with significant morbidity and mortality. While for most hepatocellular forms of drug induced hepatic injury the underlying pathophysiological mechanism is poorly understood, there is increasing evidence that cholestatic forms of drug-induced liver damage result from a drug or metabolite mediated inhibition of hepatobiliary transporter systems. There is an increasing body of evidence that genetics are a major determinant of hepatocellular transporter function. On the canalicular side, mutations in the \textit{ABCB11} and \textit{ABCB4} genes encoding BSEP and MDR3 are a well established cause of inherited cholestatic syndromes such as progressive and benign forms of familial cholestasis. Furthermore, mutations and polymorphisms in these two genes have been associated with intrahepatic cholestasis of pregnancy (ICP) and might contribute to the individual risk to develop primary biliary cirrhosis and primary sclerosing cholangitis. Patients with intrahepatic cholestasis of pregnancy or benign forms of familial cholestasis were occasionally reported to exhibit increased susceptibility to certain drugs. For instance, increased susceptibility to oral contraceptives or postmenopausal hormone replacement therapy is a frequent phenomenon in patients with ICP, while different anti-inflammatory drugs were suspected to induce cholestatic episodes in a patient with benign recurrent intrahepatic cholestasis. These observations favor the concept that a genetically determined canalicular transporter deficiency is the common pathophysiological denominator for the development of cholestasis under different extrinsic and intrinsic challenges in affected patients. In addition to such disease-causing mutations, a frequent polymorphism in \textit{ABCB11} has recently been associated with a 3-fold increased risk to develop cholestatic drug side effect under treatment with different drugs such β-lactam antibacterials, oral contraceptives, psychotropic drugs and proton-pump inhibitors. Very interestingly, this polymorphism, which leads to a valine to alanine exchange at the highly conserved position 444 of the BSEP protein has also been associated with decreased hepatic BSEP content in human liver tissue samples, offering a mechanistic explanation for this observation. It can therefore be speculated that \textit{ABCB11} and \textit{ABCB4} genetic variants found to be associated with different cholestatic conditions also predispose to the occurrence of cholestasis under treatment with certain drugs.
Variability of cholestatic liver disease in a family with 11 siblings and an ABCB4 defect

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Introduction: Defects of canalicular transporters may cause progressive liver damage. A family with 11 siblings allowed us to study the spectrum of diseases caused by a distinct pathogenic defect.

Methods: 6 of 11 siblings in a family from a population isolate in Transylvania were severely affected by a cholestatic liver disease of unknown cause. This family enabled us to perform disease linkage analysis by single nucleotide polymorphism genotyping using Affymetrix 50K Gene Chips. 9 probands (both parents, 3 severely and 4 mildly affected siblings) were included. Sequencing and further evaluation of candidate genes were performed.

Results: Based on the clinical and histopathological phenotype 6 siblings, including 3 deceased aged 5, 7 and 43 years and 3 alive with onset of disease in adulthood, were considered severely affected, with abnormal LFTs and histopathology of small-duct cholangiopathy including ductopenia. The remaining siblings and the mother were considered mildly affected with intrahepatic cholestasis of pregnancy or subtle histopathological changes. The father had no signs of liver disease. Pedigree studies revealed distant parental consanguinity, suggesting autosomal-recessive inheritance. Under this model, genotyping yielded a maximal LOD-score of 3.88 on chromosome 7q21.1-7q22, and thereby excluding other genomic regions. Detailed characterization of the 149 genes at this locus revealed that the single variant Arg788Trp (c.2362c>t) in a highly conserved domain of the ABCB4 protein co-segregated with severity of liver disease.

Discussion/Conclusion: Our results show that one, single mutation in ABCB4 causes variable forms of cholestatic and ductopenic liver diseases leading to cholestasis of pregnancy, fibrosis, cirrhosis and death in childhood and adulthood.
**ABCG5/G8 as a human risk gene for cholesterol gallstone disease**

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Genome-wide scans in inbred strains of mice have linked the genes encoding the hepatocanalicular cholesterol transporter ABCG5/G8 to gallstone formation (1). Polymorphisms in the orthologous human genes are associated with differences in serum cholesterol and plant sterol levels. We now tested these ABCG5/G8 variants for linkage and association with gallstone susceptibility in humans. Prospectively, we collected 178 Caucasian individuals with gallbladder stones or history of cholecystectomy from 84 families, 73 twins and stone-free controls, as confirmed by abdominal ultrasound. We performed nonparametric linkage (NPL) analysis in affected sib pairs (ASPs) and twin pairs, and association tests in cases and controls. Gallstones were strongly linked to the D19H variant of the ABCG8 gene (2, 3). The risk of gallstones in carriers of the 19H allele was significantly increased in cases as compared to stone-free controls (odds ratio = 3.0). Consistent with the mouse model (1), the risk variant showed an additive mode of inheritance. These findings are in line with the first genome-wide association study in a large cohort of gallstone patients from Germany (4). Of note, the D19H variant was also a susceptibility factor for gallstones in Chilean Hispanics (4) and Sorbs (5), and yet another ABCG8 variant (T400K) may affect the risk of gallstone disease in Chinese men (6).

Recent studies (7, 8) showed that the 19H allele is associated with markedly reduced serum levels of the plant sterols campesterol and sitosterol, which represent cholesterol absorption markers. Our previous study in siblings with gallstones (9) and the study by Gylling et al. (8) have also observed significantly lower serum levels of total and LDL cholesterol in 19H carriers, indicating that D19H represents a gain-of-function variant that increases ABCG5/G8-mediated removal of plant sterols and cholesterol into bile and intestine. Of note, in non-obese Chinese gallstone patients, ABCG5/G8 expression was increased significantly and correlated with the molar percentage of biliary cholesterol and cholesterol saturation index (CSI) (10). Furthermore, the 19H allele has also been associated with higher serum levels of cholesterol precursors (cholesterol, lathosterol) (8), indicating increased cholesterol biosynthesis, as has been observed in obese gallstone patients. These findings taken together suggest low intestinal cholesterol absorption and high compensatory cholesterol biosynthesis in carriers of the ABCG8 D19H variant. This might be clinically relevant, since we would predict that HMG-CoA reductase inhibitors could be particularly effective in lowering serum cholesterol concentrations (11) and biliary cholesterol levels in patients carrying the ABCG8 19H variant.

The linkage and association studies identify the cholesterol transporter ABCG5/G8 as genetic determinant of gallstone formation, i.e. LITH gene, in humans. The function of this transporter and the genetic studies taken together indicate that in gallstone-susceptible carriers of the ABCG8 19H allele, cholesterol cholelithiasis is secondary to increased hepatobiliary cholesterol secretion.
References:


ATP8B1 function and possible therapeutic interventions

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Recent studies point to a function of ATP8B1 as an inward flippase for phosphatidylserine, an aminophospholipid in cellular membranes. In vitro an increased internalization of phosphatidylserine is seen when ATP8B1 activity is enhanced. Also bile analysis of mice and men with insufficient ATP8B1/atp8b1 activity both showed an increased concentration of phosphatidylserine, probably through extraction of this aminophospholipid from the outer leaflet of the canalicular membrane. In conditions with insufficient ATP8B1 activity, both in mice and men, on electron microscopy the bile canaliculus is also found to be filled with laminar material, consistent with membrane shedding. Combined these findings point to a loss of phospholipid membrane asymmetry that renders the canalicular membrane less resistant towards hydrophobic bile salts. This loss of phospholipid asymmetry most likely causes the impaired bile salt transport, the prime characteristic of ATP8B1 deficiency. A similar mechanism might be involved in some of the extrahepatic symptoms seen in this disease, such as hearing loss and pancreatitis.

Hepatic symptomatology

In humans ATP8B1 deficiency can lead to unrelenting cholestasis, Progressive Familial Intrahepatic Cholestasis (PFIC type 1), which starts in the first few months of life. In patients with a less severe deficiency episodic cholestasis can be seen, Benign Recurrent Intrahepatic Cholestasis (BRIC type 1). Although clinically BRIC and PFIC might be considered as two entities, it is not uncommon for patients to start out with episodic cholestasis (BRIC) and to end up with permanent cholestasis (PFIC), although many patients can have frequent cholestatic episodes without ever progressing. Interestingly in mice with severe Atp8b1 deficiency no cholestasis is observed, unless challenged with hydrophobic bile salts. However, as the bile salt pool in mice is less hydrophobic than in men, it is conceivable that mice with Atp8b1 deficiency do not develop cholestasis spontaneously, and men do.

Treatment

No medical therapy is known at present that can consistently ameliorate the cholestasis in FIC1 deficiency in humans, although treatment with rifampicin may reduce itching, or even abort an episode with cholestasis in BRIC patients. The only method that gives consistent results is the diversion of the small amounts of bile salts that enter the intestine by interrupting the enterohepatic circulation. In PFIC patients the prevention of bile salt reabsorption is undertaken by ileal exclusion, or by partial biliary diversion, i.e. attaching a bowel loop to the gall bladder. Although no permanent cure is obtained, this seems to reduce the pace of progression of the liver disease and delay the inevitable development of liver insufficiency and the transplantation that is necessary subsequently, especially if the procedure is performed before extensive liver damage has occurred. In patients with BRIC, biliary diversion can also be achieved by introducing a catheter into the biliary tree by ERCP, which can be removed as soon as the cholestasis has disappeared (NasoBiliary Diversion or NBD). Liver transplantation is a good treatment for end stage liver failure due to FIC1 deficiency, although patients might suffer from severe diarrhoea post transplantation.
Bile acid abnormalities in peroxisomal disorders

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Peroxisomes play an important role in the biosynthesis of bile acids because a peroxisomal β-oxidation step is required for the formation of the mature C24-bile acids from C27-bile acid intermediates. In addition, de novo synthesized bile acids are conjugated within the peroxisome. The importance of peroxisomes in the biosynthesis of bile acids is stressed by the existence of several peroxisomal disorders associated with bile acid abnormalities. Patients with a peroxisome biogenesis disorder and patients with a deficiency of one of the following peroxisomal β-oxidation enzymes, α-methylacyl-CoA racemase, D-bifunctional protein and sterol-carrier protein X, accumulate the C27-bile acid intermediates di- and trihydroxycholestanolic acid (DHCA and THCA). The bile acid abnormalities in peroxisomal disorders can be detected easily in plasma and urine by HPLC electrospray tandem mass spectrometry. C27-bile acids have altered physical properties and as a consequence they will not function properly as bile acids. In addition, it has been suggested that the C27-bile acids intermediates are especially toxic. For these reasons, the defect in bile acid biosynthesis in peroxisomal disorders contributes to the development of cholestatic liver disease and steatorrhea.
Session VIII

Bile acids as therapeutic agents
Ursodeoxycholic acid for PSC – An update

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Ursodeoxycholic acid continues to be a popular therapy for patients with primary sclerosing cholangitis, although data supporting its use are sparse. Open-labeled studies with doses of ursodeoxycholic acid around 25 mg/kg/day show an enhanced biochemical improvement over what was seen with standard doses of 13–15 mg/kg/day. A large Scandinavian trial reported with 219 patients using a dose of 17–23 mg/kg/day failed to detect improvement in the rate of death or transplantation. The choice of rigorous endpoints, the recruitment of less than two-thirds of the expected patients, and the lower dose used may have been responsible for the negative results. Currently, there is a large ongoing study in the United States with 150 patients entered receiving a dose of 28–30 mg/kg/day with endpoints of cirrhosis, varices, cancer, liver transplantation, and death that are being followed for another three years. Information about the use of ursodeoxycholic acid lessening the risk of cancer and dysplasia in patients with PSC and colitis are also controversial with studies both supporting a reduced risk of dysplasia and carcinoma, and others not finding such an advantage at the present time. Ursodeoxycholic acid use, although a popular choice because of its safety and tolerability, is not yet supported by firm data demonstrating efficacy at endpoints other than biochemistries.
Excellent long-term survival in patients with primary biliary cirrhosis treated with ursodeoxycholic acid

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Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic liver disease of presumed autoimmune pathogenesis that usually affects middle-aged women and eventually leads to liver failure and the need for liver transplantation. Up to now, ursodeoxycholic acid (UDCA) is the specific and the only drug approved for treatment of patients with PBC. The mechanism of action of UDCA is uncertain and is probably multifactorial. Its effects are mediated by amelioration of damage to cell membranes caused by retained toxic bile acids. Moreover, UDCA improves biliary secretion of bile acids, may improve bile flow, and it has immunomodulatory properties that may reduce immune-mediated liver damage, altering and reducing inflammatory cytokine production, and the display of aberrant HLA antigens.

Since the early pilot study published in 1987, UDCA has been evaluated in several trials in patients with PBC. All these trials demonstrated favorable effects regarding improvement of biochemical cholestasis. Moreover, data from three individual studies and a combined analysis of four placebo-controlled trials have shown that treatment with UDCA delays the histological progression of the disease. Less consistent positive results have been reported concerning survival mainly because of the short duration of the trials. Thus, two meta-analyses using data collected from the first two years of UDCA therapy have suggested no therapeutic benefit of UDCA versus placebo in prolonging survival or decreasing the need for liver transplantation. These two meta-analyses pooled data from the same trials, and therefore they were not properly independent studies. Improved survival in patients with moderate or severe disease has only been demonstrated in a combined analysis of three randomized controlled trials. Furthermore, it has been proposed that the long-term outcome of noncirrhotic-stage PBC treated with UDCA is not different from the age and gender-matched population. Most of these data indicate that UDCA slows the progression of the disease and, therefore, may prolong survival. On the other hand, the response to UDCA is variable in patients with PBC, with some patients experiencing a complete normalization of liver biochemistry and others showing only minor biochemical effects. This different course of PBC patients treated with UDCA may, reasonably, have an effect on the long-term outcome of the disease.

The long-term effects of UDCA in patients with PBC have been evaluated in some recent studies. The course and survival of patients with PBC treated with UDCA has been compared with the survival predicted by the Mayo model and the estimated survival of a standardized population. One hundred and ninety-two patients (181 female) were treated with UDCA (15 mg/kg/d) for 1.5 to 14 years. Response to treatment was defined by an alkaline phosphatase decrease greater than 40% of baseline values, or normal levels after one year of treatment. The predicted survival was obtained by the Mayo model and the estimated survival was taken from the standardized matched Spanish population. During the study period 17 patients (8.9%) died or fulfilled criteria for liver transplantation and were considered as
treatment failure. Nine patients died after 3.5 to 9.1 years of treatment: four as a consequence of hepatocellular carcinoma, two because of terminal liver failure and three because of non-hepatic disease (non-Hodgkin lymphoma, gastric carcinoma and cerebral hemorrhage). Eight patients were transplanted after 2.9 to 9.1 years of treatment. Patients with treatment failure (death or transplantation) were significantly older and they had poorer baseline liver biochemistries and a higher Mayo risk score than those without treatment failure. Histological stage was also more advanced in the 17 patients who died or were transplanted. One-hundred and seventeen of the 192 patients (61%) responded to treatment, while no such apparent changes were observed in alkaline phosphatase levels in the remaining patients after one year of treatment. No significant baseline differences were found, except for bilirubin and albumin levels and the Mayo risk score, between responders and non-responders. The survival free of liver transplantation of responders was significantly higher than that predicted by the Mayo model and was similar to that estimated for the control population (p = 0.15). By contrast, the survival of the group without biochemical response was significantly lower than that estimated for the Spanish population (p < 0.001), although higher than that predicted by the Mayo model. The factors most strongly associated with death or liver transplantation were older age, higher bilirubin, lower albumin, higher prothrombin time, higher Mayo risk score, and advanced histological stage at baseline, and biochemical response after one year. Multivariate analysis identified advanced histological stage, baseline albumin lower than 38 g/l and biochemical response after one year of treatment as independent prognostic factors. Other studies demonstrated unequivocally that a large part of the benefit from UDCA therapy lies with its impact on the course of PBC in patients in early histologic stages of the disease and that this agent, when given at the early histologic stages, normalizes patient survival rates. Furthermore, recent data from a large series of PBC patients from Spain clearly indicate that survival free of transplantation is significantly longer in patients treated with UDCA than in those without UDCA, particularly at stages I to III, but not in patients with cirrhosis (stage IV).

In summary, the good biochemical response to UDCA after one year is associated with a survival similar to that estimated for the matched control population, clearly supporting the favorable effects of this treatment in PBC. The suboptimal survival observed in non-responders identifies the patients which may be included in further trials addressed to evaluate new agents or combined treatments for PBC. Moreover, further data from large series of patients with PBC treated for long periods of time with UDCA clearly sustain the favorable effects of such agent for improving survival or delaying liver transplantation in this chronic cholestatic disease.
Intravenous use of SUDCA (ursodeoxycholic disulfate) – A unidirectional bile acid, for hepatoprotection in liver transplantation and TPN-associated cholestasis

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It is generally accepted that bile secretion is the first reliable sign of hepatic function after transplantation. Bile acids are a major component of human bile and their capacity to influence bile flow and biliary lipid secretion is one of their primary functions in the liver. In previous clinical studies of assessing early graft function we have shown significant differences in donor bile-flow and bile acid secretion between normal and “marginal” grafts were related to differences in the apparent choleretic activity and bile acid composition. Furthermore allografts subjected to cold storage and subsequent reperfusion develop a degree of ischemia/ reperfusion injury due to the generation of reactive oxygen species.

Ursodeoxycholic acid (UDCA) has been shown to buffer the membrane damaging effects of hydrophobic bile acids that accumulate in cholestatic liver disease, to increase bile flow, and to have a direct protective effect on hepatocytes. As a result, UDCA has been tried in liver transplant recipients to prevent acute cellular rejection but with contradictory results. Furthermore, UDCA and tauroursodeoxycholic acid (TUDCA) have been added to the preservation solution and tested in the experimental pig liver transplant model and found to ameliorate biochemical and morphological markers of ischaemia-reperfusion damage.

Despite its hydrophilic nature, UDCA is relatively insoluble at physiological pH and therefore not ideal for intravenous use. Ursodeoxycholic acid disulfate (SUDCA) was developed as a non-absorbable and non-biotransformable bile acid in order to direct its delivery to the colon for oral use in the possible prevention of colon cancer. It is also a highly water-soluble bile acid making it ideally suited to intravenous delivery. Preclinical studies have shown SUDCA to have much greater choleretic properties than UDCA. When given intravenously, it is efficiently taken up by the liver, secreted into bile and does not undergo enterohepatic recycling like other bile acids — it is unique in being ‘unidirectional’ in its disposition.

Based on these properties and its likely cytoprotective effect we have begun to examine the potential of SUDCA in an in vivo pig liver transplantation model. In these preclinical studies we have shown that intravenous infusion of the donor liver at relatively low concentrations of SUDCA (0.25–2.0 µmol/min/100 g bw) rapidly induces an impressive dose-dependent increase in bile flow while decreasing biliary phospholipid secretion. SUDCA is completely taken up by the liver and secreted in bile unchanged resulting in a displacement of more hydrophobic bile acids. Systemic pH was unchanged despite the low pKa of SUDCA. Histological examination of the donor livers after perfusion with SUDCA shows no significant differences over the histology of saline infused controls. Studies are ongoing to evaluate the interaction of
SUDCA with liver preservation solution (HTK) after 6 hour cold ischaemia time and the hepatoprotective properties of SUDCA by examining the expression of inflammatory markers and cell death after cold ischaemic/reperfusion injury. In summary, the potent choleretic properties of SUDCA and its high water solubility suggest this is potential for intravenous use in areas of liver transplantation and prevention or treatment of TPN-associated cholestasis.

References:


Features, prognosis and management of PBC in the era of UDCA and budesonide

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Over the last two decades there have been many changes in the diagnosis, prognosis and management of patients with PBC. More patients are being recognized with early disease; most of the patients are given UDCA therapy; the requirement for liver transplantation is falling in Europe and North America (1, 2). UDCA and budesonide, the two drugs shown to be effective by controlled clinical trials are mainly active in the early phase of PBC indicating that this narrow therapeutic window should not be missed in an attempt to prevent progression towards end stage. Thus a major issue in the management of PBC is the identification of early predictors of poor prognosis in patients receiving UDCA therapy. We have repeatedly shown that serum bilirubin, histological stage and severity of lymphocytic piecemeal necrosis were strong independent predictive factors of UDCA treatment failure (3, 4, 5). However, these prognostic indices have some limitations (a) hyperbilirubinemia is only seen in less than 10% of the patients at presentation; (b) histological examination of the liver implies an invasive procedure which is not necessary in many cases for the diagnosis of PBC. Accordingly we present herein results of further studies aimed to define whether peculiar modes of presentation, presence of specific antinuclear antibodies (anti-Sp100 or Gp210) or extent of biochemical response to UDCA could help to identify subsets of patients that should be targeted very early by budesonide. Our experience with the combination of UDCA and glucocorticoids (or budesonide) in patients at risk of progression towards end stage will be also given.

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Sulphated progesterone metabolites interfere with primary human hepatocyte bile acid transport

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) usually manifests in the third trimester of pregnancy and is characterised by raised serum bile acid levels and impaired liver function. ICP is a complex disorder with genetic and endocrine factors contributing to its aetiology. Several papers have described abnormally elevated levels of sulphated progesterone (P4) metabolites in ICP. We aimed to investigate the functional impact of sulphated-P4 metabolites on primary human hepatocyte (PHH) bile-acid transport.

Methods: PHH were extracted from resected tumour-free liver tissue from male and female patients of varying age and seeded into culture plates. PHHs were pre-incubated with/without the sulphated-P4 metabolites allopregnanolone-sulphate (PM4s) and epiallopregnanolone-sulphate (PM5s) for 15 minutes prior to radio-labelled-taurocholate (3H-TC) incubation. Post-incubation, active influx and efflux were measured over time.

Results: PM4s and PM5s at 50 μM reduced the amount of 3H-TC influxed into the hepatocyte by 95% over untreated control cells. A PM5s concentration curve indicated that 3H-TC influx is most sensitive to the inhibitory effects of PM5s between the concentrations of 1–5 μM, equating to a 39% 3H-TC influx reduction. The impact of PM5s on sodium- and non-sodium dependent influx revealed that both transport components are affected by 50 μM PM5s. PHH active efflux was unaffected by 50 μM PM4s and PM5s.

Discussion/Conclusion: PHH sodium- and non-sodium dependent bile acid influx is inhibited by sulphated P4-metabolites treatment and is most marked between 1 and 5 μM PM5s. Active efflux was unaffected by PM4s/5s incubation. These data offer insights into why ICP patients develop raised serum bile acid levels.
Biliary and faecal bile acid profiles induced by *Larrea tridentata* in hamster

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Introduction: *L. tridentata*, a medicinal plant, prevents gallstone formation in hamster. Bile exhibits an increase in chenodeoxycholate and a decrease in cholate, deoxycholate and cholesterol molar percent (J Pharm Pharmacol 57: 1093, 2005). Since *L. tridentata* and its main metabolite nordihydroguaiaretic acid (NDGA) have antibiotic activity (J Ethnopharmacol 52: 175, 1996), the aim of the present work was to analyze if the plant extract and NDGA affect bile composition through hepatic or intestinal actions.

Methods: The liver of hamsters fed a fat-free-lithogenic diet was perfused with Krebs-Henseleit without (first 30 min) and with (next 20 min) *L. tridentata* ethanolic extract or NDGA (10 mg/dl). Bile was collected for 10 min periods. Faeces of hamsters fed the lithogenic diet with or without *L. tridentata* extract (0.125%) were analyzed for bile acids. Cholesterol and bile acids were determined enzymatically and bile acid profiles by TLC.

Results: In the perfused liver, *L. tridentata* extract and NDGA reduced cholesterol (p < 0.05), and increased total bile acids in bile (p < 0.05). Also, chenodeoxycholate increased and cholate decreased, significantly only with the plant extract. Total faecal bile acids decreased in animals fed the lithogenic diet, as compared with those fed a commercial diet. Feeding the plant extract reduced faecal deoxycholate by half and doubled chenodeoxycholate and cholate.

Discussion/Conclusion: These results suggest that there are hepatic and intestinal effects of *L. tridentata*, leading to a less lithogenic bile.
The hydrophobic iminosugar AMP-DNM increases biliary lipid secretion in C57BL/6 mice via FGF mediated regulation of CYP7A1

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Introduction: Treatment of ob/ob mice with the hydrophobic iminosugar AMP-DNM (N-(5'-adamantane-1'-yl-methoxy)-pentyl-1-deoxynojirimycin) ameliorates many symptoms of the metabolic syndrome. In C57BL/6 mice, AMP-DNM had no effect on glucose homeostasis but affected cholesterol metabolism. It increased liver cholesterol and decreased plasma cholesterol by about 20%.

The aim of this study was to investigate the effect of iminosugar treatment on biliary lipid secretion.

Methods: C57BL/6 mice were fed lab chow with or without 25 mg/kg/day AMP-DNM. After four weeks the bile duct of these mice was cannulated and bile was collected for 15 minutes.

Results: AMP-DNM increased biliary bile salt secretion by 200% and had similar effects on cholesterol and phospholipid secretion. The ratio of BS/Chol or BS/PL did not change significantly indicating that the primary effect was on bile salt synthesis. The expression of CYP7A1 mRNA was upregulated about threefold. To investigate the underlying mechanism and relevance for the human situation the effect of AMP-DNM on expression of CYP7A1 in HepG2 cells was studied. In vivo, fibroblast growth factor 19 (FGF19) plays a primary role in regulation of hepatic CYP7A1 expression. Pre-treatment with 10 μM AMP-DNM for 2 hours prevented the downregulation of CYP7A1 with FGF19 in vitro in HepG2 cells. Interestingly, the compound had no effect on FXR mediated downregulation of CYP7A1 by bile-salt (CDCA).

Discussion/Conclusion: Four weeks treatment with the iminosugar AMP-DNM strongly increases biliary bile salt secretion in C57BL/6 mice. In vitro experiments indicate that the effect is due to impairment of FGF19 induced down-regulation of CYP7A1.
Formation of bile canaliculi and extracellular matrix (ECM)-cell contacts

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Introduction: Apical poles of front-facing and adjacent hepatocytes form a continuous network of bile canaliculi (BC). Little information is available on the formation of this network. The role of ECM-cell interactions was evaluated using the polarized WIF-B9 line that forms simple BC between 2–3 cells.

Methods: Cells were cultured on a thin layer of various ECM components (collagen I, collagen IV, laminin, fibronectin) or on, in sandwich and inside a gel of collagen I, Matrigel, or PuraMatrix. BC formation was analyzed by immunolocalization of canalicular and tight junctional proteins, confocal microscopy and electron microscopy. BC functionality was studied by following the transport of organic anions and bile salts.

Results: The culture in Matrigel induced a rapid polarization with the formation of functional long and branched BC. In contrast, the culture on collagen IV induced a regression of WIF-B9 polarization with appearance of hudge intracellular vacuoles rich in microvilli, in which canalicular proteins (Mdr, Mrp2, ...) but not tight junctional or basolateral proteins were localized. Bile salts and organic anions were efficiently transported in these vacuoles.

Discussion/Conclusion: ECM-cell contacts modulate BC formation by WIF-B9 cells. Whereas BC formation is impeded on collagen IV, it is improved in Matrigel that allows the generation of well-developed BC reminiscent of those formed in vivo.
Fast methods to measure amidated bile acids in liver transplant patients: MS/MS characterization of side-chain analog fluorescent derivatives

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Introduction: The need for fast and complete bile acid profiles, in organ evaluation [1] and follow-up of human liver transplants [2] has been defined.

Methods: By using ESI-MS and MS/MS we have fully characterized solvents, reagents, products and side-products of reactions needed for fast and efficient fluorescent derivatization (for HPLC analysis) of side-chain analogs of amidated bile acids.

Results: The use of MS revealed that the final products of the reactions form dimer clusters with sodium, that are insoluble in the reaction solvent, thus triggering an ongoing dissolution study that identified at least one efficient solvent for the pyrene derivatives. Dissolution studies in water and acetonitrile with a novel surfactant are under way, and show that the detection limit is dependent on the 3D configuration, and aggregation state, of the dimers formed during the reaction. The MS/MS transitions/fragmentation identified can now be used to detect and quantify bile acids derivatives in the femtomole range, by HPLC-MS/MS.

Discussion/Conclusion: The use of these reactions in clinical liver transplantation may have clear advantages over other standard techniques [3]. Namely, the proposed reactions do not use carcinogenic compounds, are faster in obtaining results (under 4 h), and are less costly per sample.

References:

Transport of sulphated bile acids by canalicular ABCG2

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Introduction: ABCG2 is an ABC transporter involved in tumor multidrug resistance, whose role in healthy tissues is poorly understood. Whether ABCG2 expressed at the canalicular membrane may be involved in bile acid secretion was investigated.

Methods: The ORF of Human ABCG2 was cloned and expressed in Xenopus laevis oocytes. These cells were used in functional uptake/efflux studies. Radiolabeled tauroliothicolic-3-sulphate acid (TSLCA) was synthesized, chemically characterized and used as potential ABCG2 substrate.

Results: ABCG2 expression reduced net cell load of ABCG2 substrates, mitoxantrone and Hoechst-33342, which was restored by ABCG2 inhibition by fumitremorgin C. When rat Oatp1a1 was expressed in oocytes, the uptake of radiolabeled cholic acid (CA), glycoCA, tauroCA, taurochenodeoxyCA, tauroursodeoxyCA and taurodeoxyCA was enhanced. Co-expression of Oatp1a1 with rat Bsep, but not ABCG2, resulted in a reduction in the net uptake of these bile acids. In contrast, when TSLCA was microinjected in oocytes expressing Bsep or ABCG2, both proteins were able to export it. To investigate the effect of cholestatic steroids, estradiol 17beta-D-glucuronide was loaded together with TSLCA (cis-effect) or placed in the uptake medium whereas TSLCA was microinjected (trans-effect). In both circumstances, estradiol 17beta-D-glucuronide inhibited Bsep- and ABCG2-mediated TSLCA efflux.

Discussion/Conclusion: ABCG2 may play a role as an alternative route to BSEP for bile acid secretion into bile when sulphation of these compounds is enhanced, as happens in certain cholestatic situations. These include the lack of expression and/or function of BSEP but not BSEP inhibition by cholestatic steroids, which are also able to affect ABCG2.
The bile acids and bile alcohols of birds: Evolution gives rise to a dazzling variety of structures

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Using HPLC and MS, my laboratory has determined biliary bile salt structure in 500 avian species in order to define evolutionary relationships. Phylogenetically, birds fall into three groups. The first consists of flightless ratites; the second consists of ducks and galliformes; the third encompasses all other birds. The ratites (ostrich, emu, tinamou) are “older” than other birds; biliary bile salts consist of C_{27} bile alcohols (as sulfates). The duck/galliform branch has 5α-chenodeoxycholic acid (alloCDCA) in the oldest species, but with time this evolves to 5β-CDCA. With further time, a third hydroxy group is added to CDCA: C-1βOH (curassows); C-4βOH and C-5βOH (pheasants); C-12αOH (partridges); C-15α (swans); and C-23ROH (ducks). In the remaining large avian group, the oldest species have a novel C_{27} bile acid – 3α,7α,16α-trihydroxy (condors, woodpeckers, crows). With time, the side chain is cleaved to form the corresponding taurine conjugated C_{24} bile acid – 16α-hydroxy-CDCA (herons, pelicans, penguins, owls). There are then divergent pathways: The 16α-hydroxy group is lost to give CDCA (parrots, cormorants, tanagers) or the CDCA can undergo hydroxylation at C-12 to form cholic acid (hawks, gulls, storks). More modern species lose the C-12 hydroxy group, revert to CDCA, and then add a new third hydroxy group. This is at C-1β (pigeons, trumpeter) C-15α (plovers) and C-23R (thrushes). Because of the large number of bird species, more vertebrate species have bile acids hydroxylated at C-16 (in addition to C-3 and C-7) than at any other site on the steroid nucleus.
The evolution of bile salts in vertebrates: New findings

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Using HPLC and MS, my laboratory has determined the structure of biliary bile acids of > 1400 vertebrate species in order to define the spectrum of natural bile salts as well as to infer evolutionary pathways. Ancient species invariably have C27 bile alcohols (C27 BAlc) whereas modern species have C24 taurine conjugated (tau) bile acids (C24 BA). Three major pathways are involved in this transition: 1) direct transition of C27 BAlc to C24 tau BA (fish); 2) transition from C27 BAlc to tau C27 BA and then to tau C24 BA (reptiles); and 3) transition to a C24 glycine amidated BA followed by subsequent conversion to tau C24 BA (mammals). The 12α-hydroxy group is present on most C27 BAlc (for unknown reasons) so that side chain evolution leads to cholytaurine.

With continuing evolution, however, the 12α-hydroxy group is lost by several pathways: 1) temporary addition of a fourth nuclear hydroxyl group followed by loss of the OH group at C-12 to form new trihydroxy BA (snakes, birds, mammals; 2) loss of the C-12 OH group to form chenodeoxycholic acid (CDCA) followed by oxidation of the C-7OH group (rodents, koala) or epimerization of the C-7OH to form UDCA (bears, nutria, beaver); or 3) loss of the 12OH group to form CDCA followed by addition of a new third hydroxyl group at C-1α (marsupials), C-1β (birds); C-4β (birds, C-5β (birds), C-6α (pigs), 6β (rodents), C-15α (birds), C-16α (birds) and C-22S (fish).

My work confirms and extends the scheme of the late G.A.D. Haslewood.
Altered bile acid metabolism in childhood functional constipation: Inactivation of secretory bile acids by sulfation in a subset of patients

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Objective: An elevated concentration in the colon of chenodeoxycholic acid (CDCA) or DCA induces water secretion causing diarrhea. We hypothesized that CDCA and DCA function as endogenous laxatives. Therefore, a decrease in their proportion might cause childhood functional constipation. To test this possibility, fecal bile acid composition was determined in children with functional constipation and in non-constipated controls.

Methods: Bile acid classes were determined using electrospray ionization-single ion monitoring-mass spectrometry (ESI-SIM-MS), and individual bile acids were measured by gas chromatography-MS (GC-MS). Individual sulfated bile acids were quantified using liquid chromatography-MS (LC-MS).

Results: By ESI-SIM-MS, the proportion of DCA did not differ in constipated children (n = 73) from that in controls (n = 92), but mono-sulfated dihydroxy bile acids were greater, p < 0.05. The difference was attributable to 6 patients in the constipated group whose major fecal bile acid by LC-MS was the 3-sulfate of CDCA. By GC-MS, the bile acid profile was identical in the two groups.

Conclusions: In most children with functional constipation, the fecal bile acid profile is normal. However, there is a small subset of children whose dominant fecal bile acid is the 3-sulfate of CDCA, indicating a novel disturbance in bile acid metabolism. Such sulfation is known to abolish the secretory activity of CDCA and may contribute to constipation. We speculate that this defect arises from increased bacterial deconjugation of CDCA conjugates in the ileum, absorption of CDCA by the enteroocyte, sulfation of CDCA by sulfotransferase, and extrusion of CDCA 3-sulfate into the intestinal lumen by MRP2.
Increased serum 27-hydroxycholesterol concentration indicates an activation of LXRα and an excess of cholesterol in the human body

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Introduction: Oxysterols are essential molecules for the negative regulation of cholesterol balance in the body through the activation of LXRα. Since 27-hydroxycholesterol is formed in many tissues and is the most abundant oxysterol in human serum, the elevation of this oxysterol concentration in serum may indicate an excess of cholesterol and an activation of LXRα in the whole body. In addition, we hypothesized that the baseline serum 27-hydroxycholesterol concentration reflected to some extent a responsesness to dietary cholesterol.

Methods: In 30 healthy volunteers, serum concentrations of 27-hydroxycholesterol, lathosterol, 7α-hydroxy-4-cholesten-3-one (C4) and markers for LXRα activation i.e. plant sterols and triglycerides were measured. Then, 750 mg/day of cholesterol was added for 4 weeks to the ordinary diet, and the change of serum cholesterol concentrations was determined.

Results: The activation of LXRα upregulates ABCG5/G8, cholesteryl ester transfer protein and SREBP1c, so that serum plant sterol concentrations decrease and triglyceride concentrations increase. In fact, serum 27-hydroxycholesterol concentrations were negatively correlated with serum concentrations of sitosterol (Rs = -0.50, p < 0.01) and campesterol (Rs = -0.46, p < 0.05), and positively correlated with triglyceride concentrations (Rs = 0.71, p < 0.0005). The percent changes of the LDL cholesterol concentrations by cholesterol loading were compared between subjects with higher baseline concentrations of 27-hydroxycholesterol (≥ 80 ng/mg cholesterol) and those with lower concentrations (< 80). The former showed significantly (p < 0.05) high values (+7.4 ± 3.4%, mean ± SEM, n = 17) compared with the latter (-5.3 ± 2.7%, n = 13).

Discussion/Conclusion: Serum 27-hydroxycholesterol concentration reflects the activation status of LXRα. Subjects with high serum 27-hydroxycholesterol concentrations have excess cholesterol in the body and they are unable to adapt to a high-cholesterol diet. Inversely, a restriction of cholesterol may reduce serum LDL cholesterol more efficiently in subjects with high serum 27-hydroxycholesterol concentrations than in those with low concentrations.
ACAT2 and human hepatic cholesterol metabolism: Identification of important gender-related differences

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Objective: ACAT2 is a major cholesterol esterification enzyme specifically expressed in hepatocytes and may control the amount of hepatic free cholesterol available for secretion into bile or into HDL. This study aims to further elucidate the physiologic role of ACAT2 in human cholesterol metabolism.

Methods and Results: Liver biopsies from 17 normolipidemic and non-obese gallstone patients (female/male, 9/8) and 10 age and BMI-matched gallstone-free patients (female/male, 7/3) were collected and analyzed for microsomal ACAT2 activity, protein and mRNA expression. No difference of ACAT2 activity was observed between gallstone and gallstone-free patients. Plasma HDL-cholesterol was significantly higher in females than in males, while triglycerides and VLDL-cholesterol were significantly lower. ACAT2 activity in females was 47% of what observed in males, \( p < 0.01 \), as well as protein level, independent of gallstone disease. Moreover, activity of ACAT2 correlated negatively with plasma levels of HDL-cholesterol and with ApoAI, but not with bile acids synthesis nor with biliary cholesterol composition.

Conclusion: ACAT2 does not account for the formation of cholesterol gallstone disease, however, gallstone patients maybe a useful model for study the role of ACAT2 in cholesterol metabolism in human. In humans, a role for ACAT2 in regulating HDL-cholesterol levels seems to be present. When hepatic ACAT2 activity is low, free cholesterol may be preferentially secreted into HDL rather than into bile. Since ACAT2 activity has been proposed as pro-atherogenic, the observed gender-related differences in ACAT2 activity may also in part account for the delayed occurrence of atherosclerosis in females.
Isolation, Identification, and chemical synthesis of novel bile acids in vertebrates

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Introduction: C24 and C27 bile acids, together with C27 bile alcohols, are the predominant metabolites of cholesterol in most vertebrates. The species differences in the bile acid (alcohol) metabolism of vertebrates are of particular interest from the viewpoint of their physiological functions, as well as phylogenetic significance. We report here the isolation, identification, and chemical synthesis of the novel and major bile acids present in the gallbladder bile of the black-necked swan (Cygnus melanocoryphus), common wombat (Vombatus ursinus), and tinamou (Rhynchotus rexescens).

Methods: The bile of the vertebrates was applied to a Sep-Pak tC18 cartridge and then individual bile acids were isolated by C18 reversed-phase HPLC with an ELSD. The structures of the isolated components were elucidated by LC-MS/MS with an ESI probe and 2D-NMR techniques and confirmed by their chemical syntheses.

Results and discussion:
Black-necked swan: A major bile acid was isolated from the biliary bile and its structure was shown to be the taurine N-acylamide of 15α-hydroxychenodeoxycholic acid (3α,7α,15α-trihydroxy-5β-cholan-24-oic acid; cygnocholic acid). This trihydroxy acid was also present in the bile of tree ducks, swans, and geese.

Wombat: A major, novel bile acid in the gallbladder bile of the common wombat was identified as the taurine conjugate of 15α-hydroxylithocholic acid (3α,15α-dihydroxy-5β-cholan-24-oic acid). The wombat appears to use 15α-hydroxylation as a novel detoxification mechanism for lithocholic acid.

Tinamou: HPLC analysis of the bile acid fraction obtained from the gallbladder bile of the tinamou showed three major peaks, which were designated as compounds A (45%), B (49%), and C (3.5%). Based on the m/z values of the deprotonated molecules in the LC-ESI-MS, peak A was estimated to be a tetrahydroxy C27 bile alcohol sulfate and peaks B and C to be the taurine conjugate of C27 bile acids. Further confirmatory evidence for the detailed structures of these compounds is now being conducted.
Hepatic cytochrome p450 oxidoreductase knockout mice have altered bile formation

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Introduction: Mouse models for progressive familial intrahepatic cholestasis type 1–3 display mild phenotypes when compared to corresponding human patients, possibly caused by the bile salt (re)hydroxylation capacity of mice, which is mediated by cytochrome P450 enzymes. The aim of this study was to analyze bile formation in mice with a hepatic disruption of the Cyp 450 reductase gene (Hrn mice).

Methods: Bile formation was studied after infusion of taurodeoxycholic acid (TDC) or tauroursodeoxycholic acid (TUDC). Biliary concentrations of bile salt (BS), phospholipids (PL), cholesterol (CH) and biliary bile salt composition were measured.

Results: Endogenous bile salt output in Hrn mice was 3-fold reduced compared to wt mice, most likely due to strongly reduced bile salt synthesis. In wild type mice about 90% of the identified BS were trihydroxy BS, with 10% dihydroxy BS. In contrast, Hrn mice had only 50% trihydroxy BS with 45% dihydroxy BS and 5% monohydroxy BS. Concomitantly, the biliary excretion of PL and CH were increased in Hrn mice. Infusion of TUDC gave rise to equal biliary output rates of BS, PL and CH in wt and Hrn mice. However, infusion of TDC resulted in significantly reduced bile flow and biliary BS output in Hrn vs. wt mice, suggesting the onset of cholestasis. Conversion of TDC to TC was strongly reduced in Hrn mice.

Discussion/Conclusion: Hrn mice have a strongly reduced capacity to (re)hydroxylate bile salts and are therefore more susceptible for bile salt induced cholestasis. Hrn mice are an attractive model to study the effects of human bile salts.
Degradation of 24S-hydroxycholesterol in man is not regulated by Cyp7A1

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Objective: The conversion of cholesterol into bile acids occurs via a long cascade of enzymatically regulated oxidative processes. Our aim was to examine in humans if an upregulation of hepatic cholesterol 7α-hydroxylase (CYP7A1) by cholestyramine, a bile acid binding resin, has an effect on the degradation of brain-specific 24S-hydroxycholesterol.

Patients and methods: Six normocholesterolemic male volunteers received 4 g cholestyramine b.i.d for two weeks within an open prospective exploratory trial. Serum concentrations of lipoproteins and triglycerides were measured by enzymatic routine assays. Sterols and oxysterols were measured by gas chromatography-mass spectrometry.

Results: Total and LDL-cholesterol decreased on the average by 9.3% ($p = 0.002$) and 19.8% ($p = 0.001$) after two weeks of treatment, respectively. Absolute serum concentrations of 7α-hydroxycholesterol, a marker for bile acid production, increased 4-fold after two weeks, while 24S- and 27-hydroxycholesterol remained unchanged. Treatment with cholestyramine elevated serum levels of lathosterol, an indicator of endogenous cholesterol synthesis, by 146% ($p = 0.009$).

Conclusion: In addition to serum concentrations of total cholesterol and LDL-cholesterol, cholestyramine at a dose rate of 4 g b.i.d. causes a significant increase in the Cyp7A1 catalyzed 7α-hydroxylation of cholesterol and an up-regulation of endogenous cholesterol synthesis, as proven indirectly by an increase in serum lathosterol levels. Total serum levels of 24S- and 27-hydroxycholesterol remained unchanged indicating that an upregulation in Cyp7A1 activity is not responsible responsible for the subsequent oxidative degradation of these hydroxylated sterols.
Metabolism of side-chain modified ursodeoxycholic acid in Mdr2\(^{-/-}\) mice

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Introduction: Side chain shortened ursodeoxycholic acid norUDCA was found to be superior to ursodeoxycholic acid (UDCA) in the treatment of sclerosing cholangitis in Mdr2\(^{-/-}\) mice. We now studied metabolism of three side-chain modified UDCA.

Methods: Two-month old Mdr2\(^{-/-}\) mice were fed a diet complemented with 0.5% norUDCA, its taurine-conjugate (norUDC-tau), and 23,24-bisnorUDCA, respectively, for 4 weeks. Bile acid composition was analyzed in serum, bile, and liver homogenate using electrospray-ionization and gas chromatography-mass spectrometry (ESMS, GCMS).

Results: Total biliary bile acids in norUDCA (6.1 ± 1.3 mM) and norUDC-tau (5.7 ± 2.0 mM) fed mice did not differ from controls (5.1 ± 1.3 mM) but were lower in the bisnorUDCA (3.0 ± 0.4 mM) group. In total, both norUDCA and norUDC-tau and its hydroxylation products at C-5 and C-6 positions consisted approximately 2/3 of biliary bile acids, in the case of norUDCA in equal amounts with and without taurine-conjugation. NorUDCA and its hydroxylation products were to minor extend also excreted as glucuronidate or sulfate conjugates. In contrast, total hepatic bile acids were 4–6 times higher (p < 0.001) in norUDCA-fed mice (312 ± 16 μM) compared to control, norUDC-tau and bisnorUDCA groups (53 ± 20, 78 ± 18, 88 ± 18 μM), due to enrichment with unconjugated norUDCA and its hydroxylation products. BisnorUDCA and a bisnor-di-one as a minor metabolite became enriched in bile and liver by 6–10% only.

Discussion/Conclusion: The high enrichment of unconjugated norUDCA (and its hydroxylations products) in Mdr2\(^{-/-}\) mice liver supports the concept of intense cholehepatic shunting as the main mechanism of improvement sclerosing cholangitis.
Introduction: Covalent adduct formation of the acyl adenylate and acyl glucuronide of lithocholic acid (LCA) with proteins has been proposed as a possible explanation for the carcinogenesis and liver toxicity caused by LCA. However, no detailed study on the structure of the resulting protein-bound LCA formed in the liver has been reported. The aim of our study was to identify the cellular proteins that are chemically modified with LCA that may links via the ε-amino groups of the lysine residues in the proteins.

Methods: We generated polyclonal antibodies that recognize 3α-hydroxy-5β-steroid moiety of LCA by immunizing rabbits with immunogens in which the carboxyl group at C-24 of LCA was coupled with bovine serum albumin via a 4-aminobutyric acid and/or succinic acid spacer. The immunoaffinity approach was as follows: batch absorption followed by elution, wherein cytosolic proteins from the liver of bile duct-ligated rats were captured.

Results: The resulting antibodies largely cross-reacted with N-α-(t-butoxycarbonyl)-L-lysine-ε-LCA as well as with the amidated and nonamidated forms of LCA, thereby, the detection of LCA residues anchored on ovalbumin and lysozyme was possible by using immunoblotting. Structural analysis of the target proteins in the two-dimentional gel electrophoresis by using MALDI-TOFMS indicated that the Rab proteins of Rab-3, Rab-12, Rab-16 and M-Ras were coupled with LCA.

Discussion/Conclusion: Rab proteins are Ras-like small GTP-binding proteins that regulate vesicle trafficking pathways. The covalent binding of the Rab proteins with LCA may have affected the regulation of transport vesicles anchoring to the cognate membrane, resulting in the LCA-induced liver toxicity.
Mechanistic studies on the formation of acyl glutathione conjugate of bile acid in rat liver and its biliary excretion

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Introduction: Bile acid acyl-adenylates and acyl-CoA thioesters are reactive acyl-linked metabolites that may undergo transacylation-type reaction with the thiol group of glutathione (GSH), leading to the formation of thioester-linked GSH conjugates. In the study, we examined the hepatic transformation of these acyl-linked metabolites into GSH conjugate and their biliary excretion.

Methods: We examined the transformation of cholyl-adenylate (CA-AMP) and cholyl-CoA thioester (CA-CoA) into GSH conjugate by incubation with rat hepatic glutathione S-transferase (GST) in the presence of GSH and rat hepatic S9 fraction in the presence of GSH and ATP at 37°C for 1 or 2 h, with analysis of the reaction mixture by LC/ESI-MS/MS. Following oral administration of ursodeoxycholic acid, lithocholic acid, and [2,2,4,4-d4]-lithocholic acid to rats, biliary metabolites were analyzed by LC/ESI-MS/MS.

Results: The GST was found to catalyze the conjugation reaction in which the formation of CA-GSH conjugate formed from CA-CoA was somewhat higher than that from CA-AMP. In addition, the formation of CA-GSH conjugate along with detection of CA-AMP was demonstrated by the incubation of cholic acid (CA) with rat hepatic S9 fraction in the presence of GSH and ATP. Each of the administered bile acids was excreted as the GSH conjugate into the bile.

Discussion/Conclusion: The results of these in vitro and in vivo studies demonstrate the existence of a novel bile acid metabolism and disposition, in which bile acids are transformed to their GSH conjugates via their acyl-linked intermediary metabolites by the catalytic action of GST in the liver and then excreted into the bile.
Structure-activity relationships of AKR-type oxidoreductases involved in bile acid synthesis

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Introduction: Besides different members of the Cytochrom P450 family several oxidoreductases of the AKR, SDR and MDR families are involved in bile acid synthesis steps. Among these, the AKR enzymes 3alpha hydroxysteroid dehydrogenase (AKR1C4) and delta4-3-oxosteroid 5beta reductase (AKR1D1) carry out important reactions in the A-ring of bile acid intermediates leading to 5beta-3alpha-tetrahydrocholestanes. To understand structure-function relationships of AKR-type bile acid oxidoreductases we used X-ray crystallography and homology modelling to compare molecular properties.

Methods: Structure determination of human AKR1C4 was achieved by crystallization of recombinant enzyme in the presence of 5 mM NADPH in potassium citrate. Crystals diffracted to 2.4 Angstrom, and structure determination was achieved through molecular replacement. Substrate docking and homology modelling was achieved by using the ICM software package.

Results: Both enzymes show the typical AKR (alpha/beta) barrel motif. In close vicinity of the nicotinamide, the conserved catalytic residues Lys, His/Glu, Asp and acid/base catalyst Tyr are found. The defining residue for double bond reduction as catalyzed by AKR1D1 is Glu, involved in increasing the pKb of the catalytic Tyr residue. Steroid binding is accomplished through a mainly hydrophobic pocket that allows binding of a variety of steroid classes.

Discussion/Conclusion: The ability of closely related members of a structural scaffold to carry out consecutive steroid reactions with different chemical mechanisms indicates that this pathway has arisen by gene duplication and subsequent point mutations. The structural model for AKR1D1 helps to rationalize mutations found in cholestatic liver disease in infancy, where several mutations in AKR1D1 have been recently identified.
Unconjugated bile salts shuttle through hepatocyte peroxisomes for glycine or taurine conjugation

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Introduction: Bile acid-CoA:amino acid N-acyltransferase (BAAT) catalyses the conjugation of bile salts to glycine or taurine. Recently, we showed that rat and human Baat/BAAT are typical peroxisomal proteins, suggesting an important role for peroxisomes in reconjugation of bile salts in the enterohepatic circulation. Here, we used deuterium-labeled cholic acid (D4-CA) to study trans- and intracellular transport of CA and its glycine/taurine-derivatives in primary rat hepatocytes.

Methods: Primary rat hepatocytes were exposed to 100 μM D4-CA. In time, media and cell samples were collected and the levels of D4-CA, D4-tauro-CA (D4-TCA) and D4-glyco-CA (D4-GCA) were quantified by liquid chromatography-tandem mass spectrometry (LC/MS/MS). The subcellular accumulation of D4-bile salts was determined by digitonin permeabilisation assays and subcellular fractionation experiments.

Results: Within 24 h, primary rat hepatocytes efficiently converted 100 μM D4-CA to D4-TCA (± 23 μM) and D4-GCA (± 68 μM), which were secreted to the medium. Cellular concentrations of D4-bile salts transiently increased to 600 μM 3 hours after adding D4-CA to the medium. Low concentrations of digitonin (30–150 μg/ml) led to the complete release of D4-CA and the cytosolic protein Gapdh from hepatocytes. D4-TCA was only completely extracted with high concentrations of digitonin (≥ 500 μg/ml), similar as the peroxisomal marker proteins catalase and Baat. Moreover, purified peroxisomes contained significant amounts of D4-TCA.

Discussion/Conclusion: We established an in vitro assay to study the dynamics of bile salt conjugation and intra- and trans-cellular transport in rat hepatocytes. This enabled us to show that unconjugated bile salts shuttle through peroxisomes for taurine- or glycine-conjugation.
Increasing expression of the cholesterol binding START domain protein, StarD4, stimulates rates of bile acid synthesis and cholesterol ester formation in primary mouse hepatocytes

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Introduction: Bile acid synthesis (BAS) rates in primary hepatocytes are dramatically increased following overexpression of the mitochondrial cholesterol transport protein, StarD1. However, the role in cholesterol metabolism of other START domain proteins, like StarD4 (detected in the liver), has not been studied. The objective was to characterize StarD4 and explore its role in cholesterol/bile acid metabolism.

Methods: Human recombinant StarD4 was purified to characterize binding properties. Hepatocytes were cultured under conditions where CYP7A1 activity was undetectable. BAS rates were determined in primary hepatocytes from wild-type and cyp27A1-deficient mice following infection with recombinant adenovirus encoding StarD4, and contrasted with StarD1 and StarD5. Cholesterol ester (CE) formation was also determined using oil red staining. Acyl-coenzyme A:Cholesterol Acyltransferase (ACAT) activity was determined in microsomes isolated from mouse liver in the presence/absence of recombinant StarD4.

Results: StarD4 selectively bound cholesterol. StarD4 overexpression increased BAS rates by 4-fold in wild-type hepatocytes, but only 2-fold in cyp27A1-deficient hepatocytes (wild-type levels). StarD5 had no effect on BAS rates, whereas StarD1 had no effect in cyp27A1-deficient hepatocytes. Only StarD4 overexpression increased CE content.

Discussion/Conclusion: StarD4’s affinity for cholesterol and ability to increase BAS and CE formation, suggests that StarD4 functions as a directional intracellular cholesterol carrier. Based upon its ability to increase BAS in both hepatocyte models, StarD4 appears to not only direct cholesterol to the mitochondria like StarD1, but to the microsomes as well, with BAS mainly via the previously described mouse 25-hydroxylase microsomal pathway. Increased CE formation was further support of StarD4’s ability to transport cholesterol to microsomes.
Bile acid synthesis in man relates to plasma triglycerides, gender and presence of gallbladder but not to aging

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Introduction: Bile acids (BAs) are crucial for the absorption of fat and lipid-soluble vitamins but are also pivotal in cholesterol metabolism for both the absorption and excretion of cholesterol from the body.

Methods: BA synthesis was estimated using the serum marker 7alpha-hydroxy-4-cholesten-3-one (C4) in normal and hyperlipidemic subjects as well as during lipid-lowering therapy with resin, statin or fenofibrate.

Results: BA synthesis was 27% higher in 213 men as compared to 222 women but was unaltered during aging in contrast to serum LDL cholesterol that increased. In 244 patients with hypertriglyceridemia BA synthesis was frequently elevated whereas in 109 patients with familial hypercholesterolemia BA synthesis was normal. Treatment with cholestyramine induced BA synthesis strongly whereas fenofibrate suppressed BA synthesis while statin treatment had no effect. The level of BA synthesis was doubled in cholecystectomized subjects.

Discussion/Conclusion: The results suggest that the age-dependent increase of plasma cholesterol in humans is not due to reduced BA production. The findings of a reduced synthesis of BAs in women may explain why women have a smaller pool size of BAs, a gender difference in humans that may be of importance for why cholesterol gallstone disease predominantly strikes women.
Pathways underlying the repression of CYP7A1 by FGF19 in HepG2 cells

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Introduction: Bile salts exert negative feedback regulation of CYP7A1 expression through multiple cellular pathways. Apart from the regulatory cascade comprising FXR/SHP, activation of growth factor receptors and ensuing intracellular signaling pathways is an important determinant of CYP7A1 expression and, thus, bile salt synthesis. FGF19 is a newly identified factor produced by the terminal ileum that controls bile salt homeostasis through a partly resolved mechanism involving FGFR4 and MAPK cascades.

Methods: To study the mechanism by which FGF19 represses CYP7A1 mRNA, HepG2 cells were treated for various periods of time (0–6 hrs) with FGF19 (100 ng/ml) or CDCA (100 μM).

Results: Treatment of HepG2 cells with FGF19 resulted in significant downregulation (-50%) of CYP7A1 mRNA levels after 2 hrs, with sustained and maximal repression (-70%) occurring after 3 hrs. Similar kinetics were observed after CDCA treatment. Contrasting with a more robust and longer-lasting effect of CDCA, SHP mRNA was transiently induced after FGF19 treatment. The latter effect may be explained through observed transient induction of immediate early genes, e.g. c-Jun a known transcriptional activator of SHP, following FGF19 treatment. As determined by immunoblotting, treatment of HepG2 cells with FGF19 resulted in rapid activation of p42/44 MAPK (ERK1/2) with no effect on phosphorylation of JNK1/2 or p38 MAPK. Inhibition of MEK1/2, which prevents activation of downstream ERK1/2, attenuated the suppression of CYP7A1 mRNA by FGF19, whilst inhibition of JNK1/2 or p38 MAPK did not alter FGF19’s effectiveness.

Discussion/Conclusion: In HepG2 cells, FGF19 downregulates CYP7A1 mRNA levels via an ERK1/2-dependent pathway.
Intestinal FXR-mediated Fgf-15 production contributes to diurnal control of hepatic bile acid synthesis in chow fed mice

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Introduction: Hepatic bile acid synthesis is heavily regulated by a variety of factors, among which the intestine-derived, FXR controlled fibroblast growth factor 15 (Fgf-15). The relative importance of Fgf-15 in regulation of bile acid synthesis is unknown.

Methods: Studies were performed employing intestinal-selective FXR knockout (iFXR-KO) mice under normal dietary conditions and during challenges with taurocholic acid (0.5% wt/wt, 3 days) and a bile acid sequestrant (Colesevelam HCl, 2% wt/wt, 7 days). Bile acid kinetic parameters were examined by stable isotope dilution technique.

Results: The role of intestinal FXR-Fgf-15 signalling to hepatic Cyp7A1 expression turned out to be time-dependent. Increased Cyp7A1 was observed in iFXR-KO mice after the night period (+159% [p < 0.05] at 7 AM) but not at 7 PM when Cyp7A1 expression is maximal (+14%). Biliary bile acid secretion (+41%) as bile flow (+29%) were higher in the iFXR-KO mice in the morning. The isotope dilution technique showed that iFXR-KO resulted in an increased cholate pool size (+72%). Total bile acid synthesis was higher in the iFXR-KO mice (+57%). Biliary bile acid secretion was increased and decreased, respectively, in both groups upon feeding the bile acid-enriched diet or Colesevelam HCl. Importantly, most of the differences observed upon chow feeding were lost upon feeding of the specific diets.

Discussion/Conclusion: Intestinal FXR contributes to the control of hepatic bile acid synthesis and bile formation under normal dietary conditions, however, bile acid or bile acid sequestrant feeding leads to a situation in which the contribution of intestinal FXR in control of the process is limited.
Expression and localization of atypical PKC isoforms in liver parenchymal cells

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Introduction: Protein kinase C (PKC) isoforms are involved in bile formation. The family is divided into three classes: the classical, novel, and atypical PKCs (ζ and ι/λ). Expression and function of atypical PKCs in hepatocytes is only partially characterized.

Methods: Full-length PKCζeta and iota were cloned from human and rat liver and fused to YFP-tags. The sequence of full length rat PKCiota was not yet known and was cloned using degenerated primers and cDNA of hepatocytes. PKCζeta-YFP and PKCiota-YFP were overexpressed in HeLa or HEK cells in order to test the specificity of 6 commercially available aPKC antibodies by immunofluorescence and Western blot analysis. Subcellular localization was analyzed by cell fractionation and by immunofluorescence in isolated rat hepatocytes and liver sections.

Results: Two PKCiota antibodies were specific for PKCiota and did not react with PKCζeta. Of the four antibodies against PKCζeta only two were found to be specific in Western blot but not in immunofluorescence. Two were cross-reacting with PKCiota in both applications. Using the specific antibodies a strong canalicular staining of PKCiota in immunofluorescence studies of hepatocytes and liver sections was found. Analyzing the subcellular localization by cell fractionation and Western blotting, PKCiota was detectable in the cytoplasmic, basolateral and canalicular membrane fractions.

Discussion/Conclusion: This topology of atypical PKCs suggests a role in bile acid transport and needs to be considered in the functional evaluation of PKCζeta and PKCiota in hepatocytes. Furthermore, our study shows that the verification of antibody specificity should be a prerequisite when analyzing highly homologous protein isoforms.
Mechanisms of membrane trafficking of human organic solute transporter (hOST)

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Introduction: Human organic solute transporter (hOST) is a heterodimer composed of alpha and beta subunits. Post-translational regulation of hOST may occur by interaction with other accessory proteins that are necessary for trafficking, localization, and functional expression of the hOSTalpha and hOSTbeta.

Methods: In this study, the effects of biological reagents on the membrane localization of hOST were investigated by confocal microscopy and transport activity assay.

Results: Treatments of microtubule disrupting drugs, nocodazole and colchicine, did not effect transport activity and polarized basolateral membrane localization of hOST compared with untreated MDCK cells transfected with hOSTalpha and hOSTbeta cDNAs. In contrast, treatment with cytochalasin D (a drug inhibiting actin filament function) and bafilomycin A1 (a specific inhibitor of the vacuolar H$^+$-ATPase) significantly disrupted the polarized membrane distribution of hOSTalpha and hOSTbeta and markedly reduced the transport activity in the stably transfected MDCK cells. Treatment with Brefeldin A which causes disassembly of the Golgi complex resulted in accumulation of the hOSTalpha and hOSTbeta largely in the ER region of the stably transfected MDCK cells. The protein kinase C (PKC) inhibitor (Calphostin C), but not PKG inhibitors and PKA inhibitor (H89), interrupted the polarized membrane expression of hOST and reduced transport activity by ~40%.

Discussion/Conclusion: These studies suggest that the plasma membrane sorting of hOSTalpha and hOSTbeta is mediated by an actin and vacuolar H$^+$-ATPase associated machinery and at least partly through brefeldin A sensitive transport vesicle from ER to Golgi. PKC may involve the regulation of this membrane sorting process.
Glucuronidation of deoxycholate in rats

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Introduction: We previously reported that bile acids are extensively glucuronidated after their administration with high doses in rats. In the present study, we compared the production of the glucuronide after the administration of deoxycholate (DC) with different doses in rats.

Methods: After bile duct cannulation into male Sprague-Dawley rats, 14C-labelled DC was intravenously administered at the rate of 0.04, 0.1, 0.4 and 1 μmol/min/100 g for 60 min. Bile samples collected every 10 min were counted for radioactivity and analyzed by thin layer chromatography and bioimaging analyzer for conjugation.

Results: Maximum biliary excretion of DC conjugates was 0.030 ± 0.003, 0.083 ± 0.011, and 0.173 ± 0.021 and 0.276 ± 0.150 after DC administration at the rate of 0.04, 0.1, 0.4 and 1 μmol/min/100 g, respectively. The percentage of the glucuronide was increased (11%, 28%, 39% and 48%) and that of the taurine conjugate decreased (64%, 48%, 37%, 34%) with increased DC doses.

Discussion/Conclusion: These data indicate that, with the increased doses of DC, the capacity of taurine conjugation become plateau and the glucuronidation become enhanced to compensate it. Since the taurine conjugate and glucuronide are excreted into bile by Bsep and Mrp2, respectively, these results are considered to be reasonable from the capacity of canalicular transporters.
Caco-2 ATP8B1 knockdown cells display apical plasma membrane aberrations unrelated to FXR activation or flippase activity

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Introduction: PFIC1 and BRIC1 are hereditary cholestasis syndromes caused by mutations in the ATP8B1 gene, which encodes a putative aminophospholipid translocase. Here, we investigated the cellular consequences of ATP8B1 deficiency.

Methods: RNA interference (RNAi) was used to establish 3 independent ATP8B1 knockdown clones of the human intestinal cell line Caco-2, which were analyzed for defects in plasma membrane protein expression, sorting, flippase activity and gene expression.

Results: In differentiated Caco-2 cells, ATP8B1 was localized at the apical membranes, while this staining was undetectable in the knockdown clones. RNAi resulted in a 50-80% reduced ATP8B1 protein expression. Integral plasma membrane proteins were normally distributed in Caco-2 ATP8B1 knockdown cells. In contrast, the GPI-linked apical plasma membrane proteins alkaline phosphatase and CD13 displayed a markedly reduced expression. The aminophospholipid translocase activity in the apical membrane, on the other hand, appeared unaffected. Microarray analysis identified 250 genes that were significantly differentially expressed in all 3 clones. This list did not include Farnesoid X Receptor (FXR) or any FXR target genes, in contrast to previous data suggesting that PFIC1 patients exhibited reduced FXR target gene expression. The involvement of FXR was further excluded by quantitative RT-PCR and luciferase reporter assays. Gene Ontology analysis revealed that the list of 250 differentially expressed genes was significantly enriched in genes affecting lipid metabolism or encoding plasma membrane-associated proteins.

Discussion/Conclusion: Taken together, ATP8B1 deficiency leads to apical plasma membrane aberrations that are unrelated to FXR activation or flippase activity, but may contribute to the pathophysiology of PFIC1 and BRIC1.
Enterohepatic circulation of bile salts during essential fatty acid deficiency in mice is mainly FXR-independent

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Introduction: Cholestasis is frequently accompanied by bile salt accumulation, fat malabsorption and essential fatty acid (EFA) deficiency. In mice, EFA deficiency itself is associated with increased bile flow. Bile salts activate the nuclear farnesoid X receptor (FXR), an important bile salt homeostasis regulator. We aimed to determine the role of FXR in enterohepatic circulation (EHC) of bile salts in a mouse model of EFA deficiency.

Methods: Fxr⁻/⁻ and Fxr⁺/+ mice (C57BL/6J-129/OlaHsd) were fed EFA-deficient or control diet for 8 weeks. Afterwards, EFA status, biliary bile salts and 72 h-fat balance were determined by gas chromatography. Relevant parameters of the EHC of cholate, the major bile salt species in mice, were quantified using in vivo stable isotope methodology.

Results: EFA-deficient diet induced EFA deficiency of similar severity in Fxr⁺/+ and Fxr⁻/⁻ mice. Control diet did not induce EFA deficiency in either genotype. EFA deficiency increased bile flow (+95% in Fxr⁻/⁻ and +110% in Fxr⁺/+ mice, p < 0.05). Cholate pool size and fractional turnover rate were not significantly altered by EFA deficiency. These alterations were very similar in Fxr⁻/⁻ and Fxr⁺/+ mice. FXR deficiency increased cholate synthesis rate in mice fed either EFA-deficient or control diet. EFA-deficient Fxr⁻/⁻ mice tended to have a more hydrophobic bile salt composition (Heuman index, -0.17 vs. -0.33, p = 0.07) and higher fat absorption (78 ± 4% vs. 70 ± 4%, resp., p < 0.05), compared to their EFA-deficient littermates.

Discussion/Conclusion: The profound effects of EFA deficiency on the enterohepatic circulation of bile salts occur predominantly independent of FXR.

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Molecular associations of ABCB4 with RACK1 regulates its cellular localization and function

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Introduction: It is well established that ABCB4 translocates phosphatidylcholine across the canalicular membrane of hepatocytes and plays an important role in bile formation. However, the regulatory mechanism of the cellular localization of ABCB4 has not been established. In the present study, we identified receptor for activated C-kinase 1 (RACK1) as a regulator of ABCB4 trafficking.

Methods: Yeast two-hybrid screening was performed with cytoplasmic linker domain of ABCB4 against a human liver cDNA library. Localization of ABCB4 in non-polarized HeLa cells and polarized HepG2 cells were examined under the RACK1 down-regulated condition by siRNA. Function of ABCB4 was assessed by measuring phosphatidylcholine translocation activity in HeLa cells.

Results: RACK1 was identified as a binding partner of ABCB4 and their interaction was confirmed by co-immunoprecipitation in HeLa cells transfected with ABCB4 and RACK1. Suppression of RACK1 expression resulted in reduced protein expression of ABCB4 and its mis-localization in the cytosol of HeLa cells. Similar effect was found by suppressing RACK1 expression in HepG2 cells. Function of ABCB4 was significantly reduced by 69% when endogenous RACK1 expression was suppressed in HeLa cells. The interaction between RACK1 and ABCB4 may be specific, since ABCB1 was not co-immunoprecipitated with RACK1 in HeLa cells transfected with these two genes. Membrane surface localization of ABCB1 was also not affected by the suppression of endogenous RACK1 expression.

Discussion/Conclusion: RACK1 may have a functional significance as a regulatory cofactor of ABCB4 and its association is indispensable for the plasma membrane localization and translocation function of ABCB4.
Sodium butyrate enhances the cell surface expression and transport capacity of bile salt export pump (BSEP/ABCB11)

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Introduction: The biliary excretion of bile acids is mainly mediated by BSEP, an ATP-binding cassette transmembrane transporter located on the bile canalicular membrane. The functional suppression of BSEP causes severe cholestasis. Previously, we reported that 4-phenylbutyrate (4PBA) is a possible agent for cholestasis by increasing both the cell surface expression and the transport capacity of BSEP (Hayashi H and Sugiyama Y. Hepatology 2007; 45 (6): 1506–16). However, from the in vivo study using Sprague-Dawley rats, it appears administration of a high dose is needed for an adequate effect. In the present study, to improve its clinical application, we searched for more effective agents by screening compounds with a structural similarity to 4PBA.

Methods: To evaluate the change in BSEP-mediated transport and BSEP expression on the cell surface produced by the tested compounds, we calculated the efflux clearance of [3H]taurocholic acid across the apical membrane of BSEP-expressing MDCKII cells and carried out a cell surface biotinylation study.

Results: Compared with 4PBA treatment, BSEP-mediated transport was further enhanced by sodium butyrate (NaB). Moreover, co-administration of 4PBA and NaB was more effective than the administration of 4PBA or NaB alone. The biotinylation study suggested the enhanced BSEP-mediated transport by both NaB and 4PBA was caused by the increased BSEP expression on the cell surface.

Discussion/Conclusion: From these results, it appears NaB, like 4PBA, enhances the cell surface expression and the transport capacity of BSEP, although the mechanism of the effect by NaB is different from that of 4PBA. Further studies are underway to examine this mechanism of action.
Novel tools to study the molecular regulation of ASBT and NTCP mediated bile salt transport

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Introduction: Bile salts move across cellular membranes via specialized transporters. Na+-dependent BS import into enterocytes and cholangiocytes is mediated by ASBT, whereas hepatocytes take up bile salts mainly via the homologous NTCP. Whereas expression of these transporters by bile acid through FXR is well studied, their posttranslational regulation remains largely unsolved. Here, we present two novel complementary techniques to study ASBT and NTCP posttranslational regulation.

Methods: First, we fused epitope tags to the amino terminus of ASBT and NTCP, facing the extracellular lumen. Second, we fused the small (8 kDa) acyl carrier protein (ACP) to the amino terminus of ASBT and NTCP. This enabled the specific and covalent labelling of ASBT/NTCP at the cell surface in living cells.

Results: ASBT/NTCP tagged with ACP or antibody epitopes were mainly localized at the plasma membrane and in intracellular vesicles and its expression markedly induced functional bile salt import. Both proteins could be specifically labeled at the cell surface of living cells and were slowly internalized. We next demonstrated that bile salts increased the internalization and degradation rates of NTCP. This was confirmed using cycloheximide treatment during 10h which showed only minor NTCP degradation. However, the additional presence of bile salts resulted in a significantly decline in NTCP abundance.

Discussion/Conclusion: Together, these data imply an important role for posttranslational regulation mechanisms to modulate bile salt import activity. Furthermore, these techniques will yield unique opportunities to assess ASBT and NTCP endo- and exocytosis, plasma membrane abundance and activity in molecular detail.
Intestinal bile acid transport is inhibited by dietary calcium phosphate supplementation in healthy human adults

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Introduction: Dietary calcium phosphates are solubilised in the stomach and precipitate as amorphous calcium phosphate (ACP) in the small intestine. This ACP is capable of adsorbing bile acids (BA). In a double-blinded cross-over study we investigated the BA-binding ability of two calcium phosphates versus placebo and CaCO₃.

Methods: Thirty-one healthy adults consumed a placebo, pentacalcium hydroxytriphosphate (Penta), CaCO₃ and beta-tricalcium phosphate (beta-Tri) for four weeks each. At the end of each period, faeces were collected quantitatively for three days. BA were analysed in freeze-dried faeces and in faecal water (FW) using GC/MS.

Results: The results revealed significant BA binding. The daily excretion of total BA (sum of tLCA, LCA, iDCA, DCA, CA, CDCA and 12keto DCA) significantly increased from $639 \pm 346 \mu\text{mol/d}$ in the placebo period to $810 \pm 491 \mu\text{mol/d}$ after Penta ($p = 0.013$) and $786 \pm 394 \mu\text{mol/d}$ after beta-Tri supplementation ($p = 0.012$). This was exclusively due to an increase in the secondary BA LCA, DCA and 12keto DCA. Due to CaCO₃ supplementation there was no increase in daily total BA excretion ($710 \pm 282 \mu\text{mol/d}; p = 0.254$). In contrast, total BA concentration in FW (Placebo: $148 \pm 108 \mu\text{mol/L}$) remained constant due to Penta ($154 \pm 110 \mu\text{mol/L}, p = 0.597$) and beta-Tri supplementation ($134 \pm 85 \mu\text{mol/L}, p = 0.348$) but significantly decreased due to CaCO₃ supplementation ($124 \pm 74 \mu\text{mol/L}, p = 0.035$).

Discussion/Conclusion: In conclusion, there was a strong BA binding due to the dietary supplementation of calcium phosphates which seem to inhibit intestinal BA reabsorption. The BA-binding due to CaCO₃ supplementation was much less, affecting only those BA that are solved in FW (which amount to about 1% of excreted BA).
A biphasic response with enhanced transcription and subcellular shuttling of Y-box protein 1 to inflammatory signals regulates hepatic Mrp2 gene expression

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Introduction: Expression of hepatobiliary transporters is decreased during endotoxemia. Reduction of Mrp2 is mediated by IL-1β-dependent signals but the underlying mechanism is still unclear. YB-1 is a predominantly cytoplasmic protein which translocates to the nucleus in response to various insults. Consequently, we characterized the mechanisms of YB-1 activation and its potential role as a regulator of hepatic acute phase genes.

Methods: Liver sections from LPS-injected rats (20 h) were stained with YB-1-specific antibodies. RT-PCR quantification was performed for Mrp2, MMP-2 and YB-1. YB-1 protein was quantified from IL-1β- or TNFα-stimulated FAO cells and the localization of a CFP-YB-1-YFP fusion protein was visualized by confocal microscopy. ChIP assays and EMSA were performed analyzing YB-1 DNA-binding.

Results: In endotoxemic livers Mrp2 mRNA was down-regulated to 20%, while YB-1 mRNA expression increased 2.5-fold. Immunohistochemical staining showed a marked up-regulation and predominant nuclear localization of YB-1 protein. In FAO cells IL-1β caused an increase in cytoplasmic YB-1 protein up to 16 hours. As a rapid effect within 90 min, IL-1β-stimulation resulted in a 6-fold up-regulation of endogenous YB-1 in the nuclear compartment concomitantly with an increase in nuclear fluorescence in CFP-YB-1-YFP-transfected cells. In addition to DNA binding studies in endotoxemic rat livers, ChIP assays revealed an IL-1β-induced increase in YB-1 binding to the Mrp2 promoter in FAO cells.

Discussion/Conclusion: In conclusion, YB-1 is activated during the hepatic acute phase response by time-dependent cellular changes: an IL-1β-mediated rapid nuclear shuttling within 90 minutes and a transcriptional induction thereafter. This biphasic response now explains the IL-1β mediated suppression of Mrp2 in endotoxemic rats.
Bile acids stimulate epithelial antimicrobial defenses

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Antimicrobial peptides are important effector molecules in innate immunity and considered as endogeneous antibiotics. A central role has been indicated for these defense peptides concerning both regulation of the natural flora and in defences against pathogens. So far, the knowledge on gene regulation encoding these peptides has been limited. In the present study we further the understanding of the molecular circuits involved in the regulation of the human gene CAMP, encoding the well defined cathelicidin antimicrobial peptide LL-37. We have identified several regulatory regions in the CAMP gene and critical transcription factors but some are nuclear receptors. The nuclear receptors are recruited to the CAMP promoter upon stimulation with secondary bile acids in colon epithelial cells. Thus we show how products derived from microbes in the gut can affect expression of the CAMP gene. Our results include data on host microbe crosstalk, an essential part of gut epithelial homeostasis.

We also present a model on the mechanism how an internal enhancer operates and the proteins involved.

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Bile salt transport in liver failure

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Introduction: Acute liver failure describes a rapid deterioration of liver function in previously normal individuals. Some patients have a slower onset and are termed subacute liver failure. Bile salt secretion from the liver is under the control of specific ATP-dependent transporters in the canalicular membrane. In this study, gallbladder bile was investigated in patients with different types of liver failure.

Methods: Gallbladder bile was obtained from patients with subacute liver failure (n = 10), acute liver failure (n = 14) and a control population with colorectal cancer (n = 7). Bile salts were isolated from gallbladder bile by drying the sample onto Guthrie cards and extracting with methanol. Glycine and taurine conjugated cholate and chenodeoxycholate were separated by high-performance liquid chromatography and UV detection.

Results: There was a significant inverse correlation between total bile salt concentration and plasma bilirubin concentration in acute liver failure (r = -0.79, p = 0.0007) and in the combined liver failure population (r = -0.73, p < 0.0001). This is not a surprising finding since the two are transported by similar mechanisms. In addition, there was a significant difference between the total bile salt concentration in the subacute liver failure group compared to the control population (p = 0.04).

Discussion/Conclusion: In conclusion, there is significant variation in the ability to transport bile salts depending on the disease duration. It would appear that patients with subacute liver failure, that have had a more prolonged liver insult, have had significantly reduced bile salt transport for some time, and have correspondingly reduced bile salts in the gallbladder.
Expression of the bile acid membrane receptor TGR5 in human placenta at term

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Introduction: TGR5 is a plasma membrane-bound bile acid receptor expressed in non-parenchymal liver cells, including Kupffer cells, cholangiocytes and sinusoidal endothelial cells, where this may play a role in controlling hepatic blood perfusion. Since fetal and/or maternal bile acids may be also involved in the control of placenta perfusion through activation of TGR5, the presence and tissue localization of TGR5 in human placenta at term was investigated.

Methods: The polyclonal antibody RVLR against human TGR5 (hTGR5) was obtained in guinea pig. Its specificity was compared with commercial antibodies using Hek293 cells stably transfected with cloned hTGR5 tagged with YFP. The relative abundance of hTGR5 mRNA was determined by real-time Q-PCR. Protein localization was examined by immunofluorescence microscopy. Antibodies against trophoblasts (CK-7), endothelial cells (VeCad), fibroblasts (αSMA) and macrophages (CD163) were used to identify different types of placenta cells.

Results: In human term placentas, TGR5 mRNA levels were higher than the levels found in sinusoidal endothelial cells and Kupffer cells of rat liver and equal to the amounts found in human gallbladders. In contrast to RVLR, none of the tested commercially available antibodies were specific for hTGR5. Using RVLR, hTGR5 was detected in placenta. The immunoreactivity was stronger in fetal macrophages or Hofbauer cells than in trophoblast and BeWo cells.

Discussion/Conclusion: The high hTGR5 expression in Hofbauer cells, localized between trophoblasts and endothelial cells, suggests that TGR5 may play a role in bile acid-induce signaling in these macrophages. Further studies are needed to elucidate TGR5 function in the placenta.
An investigation into the effect of bile acids on Golgi morphology in a model of oesophageal cancer

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Introduction: Bile acids such as deoxycholic acid (DCA) have been implicated in colon cancer and Barrett’s oesophagus. They have been found to stimulate a number of intracellular signalling cascades mediated by a range of molecules. In contrast, data suggests that ursodeoxycholic acid (UDCA) acts to reduce colorectal dysplasia and cancer occurrence in patients with inflammatory bowel disease. The purpose of this work was to investigate the effect of bile acids on oesophageal cell morphology, focussing on changes induced in the Golgi apparatus.

Methods: An assay to investigate the effects of DCA and UDCA on the Golgi has been developed and adapted to high content analysis (HCA).

Results: We have demonstrated that DCA causes the Golgi to fragment throughout the cell. UDCA has been found to inhibit this fragmentation. Furthermore, we have shown that UDCA induces translocation of the glucocorticoid receptor (GR) to the nucleus in our system. The role of the GR in mediating the protective effects of UDCA on the Golgi was investigated. When cells were treated with the GR antagonist, mifepristone, UDCA was no longer able to inhibit DCA induced fragmentation indicating that UDCA mediates it effects via a glucocorticoid receptor-regulated pathway.

Discussion/Conclusion: The Golgi is involved in post-translational modification of proteins and membrane trafficking. Disruption of these processes may lead to abnormal intracellular signalling resulting in carcinogenesis. This represents a novel target for therapeutic intervention with UDCA. It is hoped to synthesise a number of analogues of UDCA with a view to making a more potent cytoprotectant for chemoprevention.
Bile acids influence muscarinic receptor signalling pathways in cardiomyocytes

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) can be complicated by intrauterine death, fetal distress and spontaneous pre-term labour. The mechanism of intrauterine death in ICP is poorly understood. Previous studies have demonstrated an abnormal fetal heart rate in ICP pregnancies. We hypothesised that raised fetal serum bile acid levels cause arrhythmogenic fetal death and demonstrated that addition of taurocholate to rat neonatal cardiomyocytes in culture causes abnormal contraction and altered calcium dynamics. There is evidence that bile acids bind to muscarinic receptors. We aimed to study the involvement of the M2 muscarinic receptor and its downstream targets in bile acid-induced arrhythmia using rat neonatal cardiomyocytes (RNCM) as a model of the fetal heart.

Methods: Scanning Ion Conductance microscope was used to measure contraction. Western blot was used to examine protein expression.

Results: We showed that the addition of 1 mM taurocholate to RNCM caused a 73% reduction of contractions per minute (SEM = 1.33; p < 0.001). Pre-incubation with atropine (non-selective muscarinic receptor blocker), methoctramine (specific M2 muscarinic receptor blocker) and LNMA (Nitric oxide synthase blocker) abolished the effect of 1 mM taurocholate-induced dysrhythmia in RNCM. ERK1/2 is a known downstream target of the muscarinic receptor. Western blot analyses of RNCM treated with taurocholate for 1 to 4 hours showed an increase in phospho-ERK1/2 levels when compared to untreated control cells.

Discussion/Conclusion: The data indicates that the muscarinic receptor, ERK1/2 and nitric oxide are involved in taurocholate-induced arrhythmia and may offer insights into ICP intrauterine death.
Cholic acid, a key factor for the development of atherosclerosis?

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Introduction: The primary bile acid, cholic acid (CA), strongly promotes intestinal absorption of cholesterol, and represents the most important endogenous FXR ligand in mice. Mice deficient for Cyp8b1 are unable to synthesize CA, have reduced cholesterol absorption and an increased bile acid synthesis. The aim was to investigate whether absence of endogenous CA production protects from atherosclerosis development.

Methods: A double knockout mouse was created by cross-breeding Cyp8b1-deficient mice with ApoE-deficient mice. Groups of ApoE-/- Cyp8b1-/- mice and ApoE-/- mice were fed a diet containing 0.2% cholesterol for 2 weeks or 5 months, thereafter the extent of atherosclerosis was monitored as well as expression levels of selected liver genes and lipoprotein plasma profiles.

Results: Compared to ApoE-/- mice, double-knockout mice displayed significantly less atherosclerotic lesions in their aortic roots and 50% lower levels of cholesterol and triglycerides in plasma VLDL and LDL fractions. These mice absorbed 50% less intestinal cholesterol. Similar to Cyp8b1-/- mice they had increased mRNA levels of enzymes involved in the synthesis of bile acids and cholesterol.

Summary: The atheroprotective effect in mice devoid of CA may be related to an increased bile acid synthesis and alterations in the handling of intestinal and hepatic cholesterol, causing reduced plasma levels of cholesterylester-rich lipoproteins.
Effect of tauroursodeoxycholate on biliary BSP excretion and hepatic Mrp2 immunostaining in bile duct-ligated rats

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Introduction: Hepatic transporters such as Mrp2 and Bsep are known to be down-regulated in obstructive jaundice. In the present study, the effect of tauroursodeoxycholate (TUDC) on the biliary excretory maximum of BSP, the glutathione conjugate of which is an Mrp2 substrate, was studied in bile duct-ligated (BDL) rats for 3 days (BDL). Furthermore, the effect of TUDC on the Mrp2 expression in BDL rats was also studied by immunohistochemistry.

Methods: After bile duct cannulation, BSP was intravenously administered at the rate of 0.2 μmol/min/100 g, and TUDC was infused at the rate of 0.8 μmol/min/100 g. Immunostaining of Mrp2 was performed by using monoclonal antibody, M2II-6.

Results: Biliary excretory maximum of BSP (nmol/min/100 g) was markedly decreased in BDL rats (11 ± 3) compared to controls (87 ± 9), and was relieved to 38 ± 19 by the coadministration of TUDC. Immunostaining of Mrp2 was decreased only periportal areas of the liver (2.67 ± 0.23 vs. 4.35 ± 0.62 in sham-operated rats by relative intensities of an image analyzer), which was relieved by TUDC (4.02 ± 0.89).

Discussion/Conclusion: In conclusion, the recovery of the biliary excretion of BSP-glutathione by TUDC in BDL rats is considered to be due to the partial recovery of the Mrp2 function by TUDC.
Effects of long term hydrophilic bile acid therapy on in vitro contraction of gallbladder muscle strips in patients with cholesterol gallstones

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Introduction: Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid which has become the drug of choice for the dissolution of cholesterol-rich gallstones as it is effective in dissolving cholesterol gallstones in selected patients. Impaired gallbladder emptying has been associated with gallstone disease and the effects of bile acid therapy for gallstone dissolution have become a contentious issue. We have evaluated UDCA therapy on the in vitro contraction of gallbladder smooth muscle strips from cholesterol gallstone patients.

Methods: The contraction forces of gallbladder smooth muscle strips from 28 patients with cholesterol gallstones treated with UDCA and 14 untreated patients were compared. The strips were stimulated with increasing concentrations of cholecystokinin-8 (CCK-8).

Results: Although the contraction forces developed in response to CCK-8 were higher in strips from specimens of UDCA treated patients compared to untreated patients, longer treatment periods (6-week), caused more contraction responses than short treatment period of 3-week.

Discussion/Conclusion: Six-week UDCA treatment caused an increase in contraction of muscle strips from patients with cholesterol gallstone when compared to shorter administration or controls. We suggest that extending UDCA treatment period may cause more effective contractions in the gallbladder, and thereby increase the rate of response to treatment.
Taurodeoxycholate enhances xanthine oxidase-mediated reactive oxygen species generation and thereby reduces cell viability in human hepatic stellate cells

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Backgrounds: Chronic cholestasis is clinically known to cause the development of liver cirrhosis. Under these conditions, hepatocytes, as well as cholangiocytes and hepatic stellate cells (HSCs) are exposed to high concentration of bile acids (BAs). As a result, hepatocytes undergo necrotic and apoptotic cell death, whereas HSCs become activated and transformed into major fibrogenic cells that contribute to collagen accumulation in the liver. Our preliminary experiment has revealed that certain BAs induce the generation of reactive oxygen species (ROS) in both immortalized human hepatocytes and cholangiocytes, resulting in the alteration of cell proliferation and function.

Aims: To characterize the pathogenesis of cholestatic liver fibrosis, we aimed to investigate the effect of BAs on ROS generation and proliferation in HSCs.

Methods: Human HSC cell line LX2 (kindly provided by Dr. Scott Friedman) was incubated in media supplemented with 200 μM of following BAs: taurochenocholate (TC), taurodeoxycholate (TDC), taurochenodeoxycholate (TCDC), tauroursodeoxycholate (TUDC), and glycochenodeoxycholate (GCDC). MTT assay was utilized to examine the cell proliferation following 72 hr of exposure to BAs. The influence of BAs on cellular ROS generation was quantified spectrophotometrically using a 2,7’-dichlorofluorescein diacetate (DCF-DA) to detect the liberation of DCF. The population of apoptotic cells was analyzed with a FACS Calibur instrument using annexin V-FITC/PI staining. To assess the site of ROS generation induced by BAs, inhibitory effect of following chemicals were tested: DPI (an NADPH oxidase inhibitor), KCN (an inhibitor of mitochondrial ROS generation), allopurinol (an inhibitor of xanthine oxidase), and indomethacin (an inhibitor of cyclooxygenase).

Results: Among the BAs tested in this study, TDC has a prominent effect on the biological property of LX2. TDC significantly reduced cell proliferation by 25%, which was in parallel with increased cellular generation of ROS by 77%. The reduced proliferation of LX2 by TDC was consistent with a profound increase of apoptotic cells by 44%. TDC-induced ROS generation was suppressed by the presence of allopurinol (-35%) and antioxidants (-85%), suggesting xanthine oxidase play a pivotal role in these ROS generation.

Conclusion: We conclude that the stimulation of TDC induces ROS generation mainly via xanthine oxidase in HSCs, resulting in cell apoptosis and reduced cell viability. Considering the previous report that BA species other than TDC stimulate the proliferation and activation of HSCs via ROS generation, we speculate that specific BAs exhibit distinct biological activities against HSCs.
Liver specific activity of FGFR4 depends on its glycosylation status

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Introduction: De novo bile salt synthesis needs to be tightly regulated in order to maintain optimal bile flow and prevent cholestasis. Lately, it has been shown that bile salts induce production of FGF19 in the intestine that can suppress expression of CYP7A1 and de novo bile salt synthesis in the liver. FGFR4, the hepatic receptor for FGF19, seems to be differentially glycosylated in the liver compared to other tissues. Therefore we wanted to investigate the role of glycosylation in the hepatic activity of FGFR4.

Methods: HepG2 and 293T HEK transfected cells were used as a model system to study the effect of various inhibitors on the glycosylation and activity of FGFR4.

Results: Two glycoforms of FGFR4 are present in the cell, the core glycosylated form produced in the Endoplasmic Reticulum and the terminally glycosylated form produced in the Golgi. HepG2 cells express primarily the terminally glycosylated form while FGFR4-transfected HEK cells express mainly core glycosylated FGFR4, meaning that there are liver specific factors that increase the cellular abundance of terminally glycosylated FGFR4. The balance between the two glycoforms is essential in FGF19 signalling since inhibitors that block terminal FGFR4 glycosylation, block also FGF19 activity. By co-transfections, we identified liver specific co-factors that are responsible for skewing the balance towards terminally glycosylated FGFR4 also in HEK cells.

Discussion/Conclusion: Terminally glycosylated FGFR4 is essential for FGF19 signalling. Hepatic co-factors that increase the abundance of this glycoform could justify the liver specific activities of FGFR4.
Prebiotic oligosaccharides and the enterohepatic circulation of bile salts in rats

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Introduction: Human milk contains prebiotic oligosaccharides which stimulate the growth of intestinal bifidobacteria and lactobacilli. It is unclear whether the prebiotic capacity of human milk contributes to the larger bile salt pool size and the more efficient fat absorption in infants fed human milk compared to formula.

Methods: We determined the effect of prebiotic oligosaccharides on bile salt metabolism in rats. Rats were fed a control diet, or an isocaloric diet containing a mixture of galactooligosaccharides (GOS), long chain fructooligosaccharides (lcFOS) and acidified oligosaccharides (AOS) for three weeks. We determined synthesis rate, pool size and fractional turnover rate (FTR) of the primary bile salt cholate using stable isotope dilution methodology. We quantified bile flow and biliary bile salt secretion rates through bile cannulation.

Results: Prebiotic intervention resulted in significant changes in fecal and colonic flora: the proportion of lactobacilli increased with 344% (p < 0.01) in colon content and with 139% (p < 0.01) in feces compared to the control group. The number of bifidobacteria also increased with 366% (p < 0.01) in colon content and with 282% in feces after the prebiotic treatment. Furthermore, pH in both colon and feces decreased significantly with respectively 1.0 and 0.5 pH point. However, despite this alteration of intestinal bacterial flora, no significant effect on relevant parameters of bile salt metabolism and cholate kinetics was found.

Discussion/Conclusion: The present data in rats do not support the hypothesis that prebiotics naturally present in human milk contribute to a larger bile salt pool size or altered bile salt pool kinetics.
The hydrophobic iminosugar AMP-DNM stimulates biliary lipid secretion but has no effect on maximal bile salt output

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Introduction: The hydrophobic iminosugar AMP-DNM (N-(5'-adamantane-1'-yl-methoxy)-pentyl-1-deoxynojirimycin) is a potent inhibitor of glucosylceramide synthase, a key enzyme in glycosphingolipid synthesis. Two weeks treatment with the compound reduced the hepatic glycosphingolipid content by about 40%. In C57BL/6 mice AMP-DNM did not affect glucose homeostasis but induced a surprising increase in biliary lipid secretion. It was the aim of this study to investigate whether this is due to increased activity of the canalicular ABC-transporters.

Methods: C57BL/6 mice were fed for two weeks with AMP-DNM at a dose of 100 mg/kg/day. At day 14 the bile duct of the mice was cannulated via the gallbladder and bile was collected for 90 min. Subsequently, tauroursodeoxycholic acid was infused in stepwise increasing concentration.

Results: Confirming earlier results two weeks treatment with 100 mg/kg AMP-DNM increased bile flow and secretion of BS by about 100%. Expression of Abcb11 increased 50% whereas no change of expression in Abcb4 or Abcg5/Abcg8 was observed. Interestingly, bile salt independent flow was significantly decreased in the AMP-DNM treated animals indicating that the increased bile flow was caused by the increased BS secretion only. Infusion of TUDC up to 2400 nmol/min/100 g body weight stimulated BS output to a similar extent in both control and AMP-DNM treated animals. Maximal biliary cholesterol secretion decreased from 12.7 ± 3.5 to 7.7 ± 3.5 nmol/min/100 g body weight indicating decreased activity of Abcg5/Abcg8.

Discussion/Conclusion: AMP-DNM strongly increased bile salt secretion in C57BL/6 mice but this was not due to increased activity of Abcb11. We speculate that an increase in the BS pool due to increased activity of BS synthesis accounts for the observed effects.
Hepatic expression of nuclear receptors and biliary transporters in human cholesterol gallstone disease

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Introduction: The molecular mechanisms underlying cholesterol cholelithiasis are largely unknown. Aim of this study was to analyze the hepatic expression of a number of genes involved in bile acid metabolism and biliary transport in human cholelithiasis.

Methods: Surgical liver biopsies were obtained in 16 patients with untreated cholesterol cholelithiasis and 13 gallstone-free subjects; mRNA levels of cholesterol 7alpha-hydroxylase, related nuclear receptors and coactivators and biliary transporters, were assayed by quantitative real-time RT-PCR.

Results: No differences were detected in the expression of most genes involved in bile acid synthesis; PPAR-gamma coactivator 1alpha (PGC-1alpha), a coactivator of cholesterol 7alpha-hydroxylase involved in insulin sensitivity and energy balance, was less expressed (p < 0.05) in gallstone subjects. Expression of biliary cholesterol transporters ABCG5 and ABCG8 was significantly increased in gallstone patients. Conversely the expression of the bile salt export pump (BSEP) was reduced, and was linearly correlated with PGC-1alpha. Finally the HOMA-IR index, a marker of insulin resistance, was higher in gallstone patients and correlated inversely with PGC-1alpha expression.

Discussion/Conclusion: Specular alterations of the expression of cholesterol and bile acids transporters occur in human cholelithiasis. PGC-1alpha seemingly plays a preventive role; the relationship with HOMA-IR suggests a link with insulin resistance. Such effects might take place via interaction with FXR and target genes, such as BSEP. These findings may help to identify novel molecular targets for the prevention and/or treatment of gallstone disease.

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Mechanism of suppression of human NTCP gene expression by inflammatory cytokines

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Introduction: NTCP is the chief hepatic uptake system for bile acids from portal blood. We have recently elucidated, how human NTCP (hNTCP) gene expression is suppressed by elevated bile acids, a situation observed in cholestasis. Cholestasis is frequently associated with inflammation. Here we investigate, whether inflammatory cytokines decrease hNTCP expression, as previously shown for rats. The rat Ntcp promoter elements mediating cytokine-mediated suppression are not conserved in humans, thus a different mechanism must operate.

Methods: Changes in mRNA and protein expression were measured by TaqMan PCR and immunoblotting, respectively. Luciferase-linked promoter activities were assayed in transient transfections.

Results: TNF-alpha and IL-1beta treatments decrease NTCP expression in human liver-derived cells and primary hepatocytes. Having previously shown that the glucocorticoid receptor (GR) induces hNTCP mRNA expression and this induction is suppressed by bile acids, we demonstrate that GR-maintained hNTCP expression is decreased by the two cytokines. The hNTCP promoter is suppressed by TNF-alpha or IL-1beta via a region between nucleotides -87/-36, which contains a binding motif for the transcription factor CCAAT/enhancer-binding protein-beta (C/EBPbeta). The C/EBPbeta gene encodes several isoforms through proteolysis and/or alternative translation initiation. A 16 kDa isoform, the liver inhibitory protein (LIP) contains no transactivation domain, and acts in a dominant-negative manner. We show that LIP expression is increased in liver-derived cells upon treatment with the cytokines. Exogenously expressed LIP suppresses, while full-length C/EBPbeta activates, hNTCP promoter activity.

Discussion/Conclusion: Inflammatory cytokines may downregulate hNTCP expression by inducing LIP protein expression. Human NTCP expression is determined by the interplay between glucocorticoids, bile acids, and cytokines.
FXR activation reduces TNFα-induced inflammatory signaling

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Introduction: Inflammatory bowel disease (IBD) constitutes a chronic inflammatory disorder of the gut caused by genetic and environmental factors leading to activation of immune responses and loss of tolerance to enteric bacteria. The farnesoid X receptor (FXR) is highly expressed in the ileum, and is activated by bile salts thereby regulating transcription of genes involved in bile salt homeostasis. Furthermore, Fxr activation prevents small intestinal bacterial overgrowth in mice, suggesting a possible role for FXR in protection against inflammation and IBD.

We investigated whether FXR activation modulates TNFα-induced inflammatory signalling in vitro.

Methods: Differentiated colon-carcinoma-derived HT29 cells were treated with synthetic FXR ligand GW4064 (24h) and/or TNFα (6 h). RNA was isolated and analysed by Q-PCR.

Results: GW4064 treatment significantly increased expression of FXR target genes IABP and FGF19 (16- and 4-fold, respectively), whereas TNFα treatment increased expression of inflammatory markers IL-8 and NFkB-1b (800 and 2.5-fold, respectively). Co-treatment with TNFα resulted in attenuation of the GW4064-induced increase of IABP and FGF19 expression (37.5% and 13%). In addition, the TNFα-induced increase in IL-8 and NFkB-1b expression was markedly reduced by GW4064 (19% and 40%). In undifferentiated HT29 cells, in which FXR expression is absent, TNFα-induced IL-8 and NFkB-1b expression did not change upon GW4064 co-treatment, strongly suggesting that attenuation of pro-inflammatory marker expression in differentiated cells is FXR-dependent.

Discussion/Conclusion: In conclusion, TNFα decreases FXR activation and FXR activation decreases TNFα-induced inflammatory signaling in HT29 cells. Further research is needed to explore whether pharmacological modulation of FXR activity is beneficial in treatment of inflammation and IBD.
The role of the nuclear receptors FXR, PXR and CAR in placental bile acid homeostasis

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy specific liver disorder characterised by raised maternal serum bile acid levels and associated with adverse fetal outcome. The aetiology of ICP is complex and not fully understood, but the fetal complications are likely to result from an accumulation of bile acids in the fetal circulation. This study has been undertaken to investigate the mechanisms governing placental transfer of bile acids in normal and cholestatic pregnancies.

Methods: Samples of villous trophoblast from 6 ICP and 7 normal pregnancies, and of 3 human livers were collected and preserved in RNA later. Explant cultures treated with chenodeoxycholic acid or lithocholic acid were prepared from 4 control placenta. Total RNA was extracted using Trizol, and reverse transcribed to cDNA. Quantitative real-time PCR was performed using SYBR Green. Target gene mRNA abundance was calculated from a standard curve and normalised to L19.

Results: The expression of FXR, PXR and CAR, the nuclear receptors responsible for hepatic bile acid homeostasis and several FXR target genes (SHP, MDR3 and BSEP) was found to be very low in control placenta, and unaffected by cholestasis. Furthermore, the expression of these genes in placental tissue could not be induced in vitro by exposure to bile acids.

Discussion/Conclusion: These results suggest that FXR, PXR and CAR are expressed at very low levels in normal and cholestatic placenta, and are unlikely to play a major role in the regulation of genes responsible for bile acid handling by the placenta at term.
The human small heterodimer partner (SHP) promoter contains independent and unconventional 9-cis retinoic acid- and bile salt-responsive elements

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Introduction: The small heterodimer partner (SHP) is an important transcriptional regulator of bile salt homeostasis. SHP expression is regulated by the bile salt sensor, farnesoid X receptor (FXR). FXR functions as a heterodimer with the retinoid X receptor alpha (RXRa). Recently, we found that the ligand for RXRa; 9-cis-retinoic acid (9cRA) differentially affects chenodeoxycholate (CDCA)-induced expression of FXR target genes. 9cRA stimulates CDCA-induced expression of SHP, while repressing bile salt export pump (BSEP) expression. Here, we studied the molecular mechanism of the co-stimulatory effect of CDCA and 9cRA on SHP expression.

Methods: hFXR/RXRα-transfected DLD-1 cells (human colon cell line) were cultured in the presence/absence of CDCA and/or 9cRA. A luciferase reporter plasmid containing deletion mutants of a 579-bp hSHP promoter element were used to determine the location of CDCA and/or 9cRA responsive elements. Q-PCR and luciferase reporter assays were used to quantify mRNA levels of FXR target genes and the hSHP promoter activity, respectively.

Results: 9cRA and CDCA act synergistically on activation of hSHP transcription. Remarkably, CDCA-induction was still observed with an hSHP promoter element, which was 5'-truncated up to position -122 and lacks the previously identified FXRE at position -278 to -291. The region between -122 and -68 was required for CDCA (and also GW4064)-dependent activation, even in the presence of the -303 to -122 upstream hSHP promoter sequence, including the previously identified FXRE. 9cRA dependent activation was lost when truncating the hSHP promoter to -276.

Discussion/Conclusion: Human SHP expression is regulated through novel and spatially separated FXR/CDCA- and RXRα/9cRA-response elements.
Enterohepatic cycling of ezetimibe-glucuronide depends on Abcc3 and Abcc2

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Introduction: Ezetimibe lowers plasma cholesterol levels by inhibiting the uptake of cholesterol in the intestine. Due to extensive enterohepatic circulation of ezetimibe, relative low doses are required to be effective. In blood and bile the majority of ezetimibe is present as a glucuronide-conjugate, which is formed in the enterocyte. Abcc2 and Abcc3 are ABC transporters, which are expressed in both liver and intestine and capable of transporting glucuronidated compounds. The aim of this study is to investigate the contribution of Abcc2 and Abcc3 in the enterohepatic cycling of ezetimibe-glucuronide (Ez-gluc).

Methods: Transport studies were performed in membrane vesicles from ABCC2 and ABCC3 protein containing Sf21 insect cells. Furthermore, intestinal tissues from wild-type and Abcc3-/− mice were used to study vectorial transport in an Ussing chamber setup. Finally, biliary excretion of Ez-gluc was measured in vivo after duodenal delivery of ezetimibe in wild-type, Abcc2−/− and Abcc3−/− mice.

Results: ABCC3- and ABCC2-mediated transport of [3H]Estradiol-17β-glucuronide was dose dependently inhibited by Ez-gluc. Half maximal inhibition was achieved at Ez-gluc concentrations of 30 μM and 7.5 μM for ABCC2- and ABCC3-mediated transport of [3H]Estradiol-17β-glucuronide, respectively. In the Ussing chamber Ez-gluc recovered from the basolateral side was significantly reduced in duodenal (45-fold), jejunal (4.3-fold) and ileal (4.3-fold) tissue of Abcc3−/− compared to wild-type mice. Biliary excretion of Ez-gluc was significantly reduced in Abcc2−/− (1.8-fold) and Abcc3−/− (2.9-fold) compared to wild-type mice.

Discussion/Conclusion: These data demonstrate that basolateral excretion of Ez-gluc in the enterocyte strongly depends on Abcc3 activity, while hepatobiliary excretion of Ez-gluc is, in part, mediated by Abcc2.
Mouse pregnancy causes bile acid overload by similar mechanisms to Fxr deficiency

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Introduction: During gestation, serum bile acid (BA) levels rise and in predisposed individuals, reach pathological concentrations resulting in intrahepatic cholestasis of pregnancy (ICP). We have reported functional variation in FXR, the principle BA regulator, in ICP.

Methods: We aimed to study the mechanisms of BA regulation during pregnancy by comparing gestation to two other models of BA overload in mice; cholate (CA) feeding and Fxr deficiency.

Results: Serum bile acids are increased in pregnant wt (7-fold, p = 0.056) and non-pregnant Fxr-/- mice (10-fold, p < 0.05) compared to wt controls. Hepatic microarray profiling shows increased expression of genes involved in BA biosynthesis (Cyp7a1, Cyp8b1) and decreased expression of genes involved in basolateral import (Oatp1, Oatp8, Ntcp), canalicular export (Bsep, Mrd3, Atp8b1 [FIC1], Abcg5/8) and the nuclear receptor Shp in both these groups. These are plausible explanations for the rise in serum BA in pregnant and Fxr-/- animals. In contrast to pregnancy and Fxr-deficiency, wt mice fed a CA-supplemented diet (14-fold increase in serum BA, p < 0.01) displayed decreased expression of the key genes involved in BA biosynthesis (Cyp7a1, Cyp8b1) and increased expression of genes involved in basolateral import (Oatp1) and canalicular export (Bsep, Mrd3, Atp8b1 [FIC1], Abcg5/8).

Discussion/Conclusion: These data demonstrate that gene expression changes induced by CA feeding are a consequence of raised serum BAs and that pregnancy causes gene expression changes, similar to those of Fxr-/- animals, which result in raised serum BA. Since most of the genes involved are regulated by Fxr, lowered Fxr activity may be causative for pregnancy-induced BA overload.
25-Hydroxycholesterol-3-sulfate (25HC3S) regulates lipid metabolism by activation/inactivation of nuclear orphan receptors in hepatocytes and macrophages

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Introduction: We recently identified a novel oxysterol sulfate, 25-hydroxycholesterol 3-sulfate (25HC3S), as an important regulatory molecule. Nuclear orphan receptors, such as LXR, SREBPs, and PPARs, are key transcriptional regulators of lipid metabolism. SREBPs directly regulate expression of 32 genes involved in lipid metabolism. We now have studied the effects of 25HC3S on the nuclear receptor activation/inactivation.

Methods: 25HC3S was chemically and enzymatically synthesized. Effects of 25HC and 25HC3S on expression and activation/inactivation of LXR, SREBPs, PPARs, and their targeting genes involved in lipid metabolism was compared and evaluated in human macrophages and primary rat hepatocytes.

Results: Addition of 25HC3S to the cell cultures markedly decreased nuclear LXR protein levels in 1 hr, followed by dose- and time-dependent decreases in SREBP-1 proteins and mRNA levels (~4-fold) as well as Cyp7A1 mRNA (~6-fold). This suggests that 25HC3S may complex with LXR to prevent its activation, thereby removing a key stimulus of SREBP-1 transcription. 25HC3S administration also led to a decrease in mature SREBP-1 accompanied by an increase in SREBP-1 precursor and subsequently decreased the expression of a number of SREBP-1-responsive genes including acetyl CoA carboxylase-1 (ACC-1) and fatty acid synthase (FAS). 25HC, the precursor of 25HC3S, had opposite and competitive effects, increasing SREBP-1 and FAS mRNA levels. Furthermore, 25HC3S increases nuclear PPARγ and decreases nuclear SREBP-2 levels and expression of its targeting genes, HMGR and LDLR, in the macrophages.

Conclusion: This work implies that sulfation of 25HC dose more than simply inactivation of 25HC. It provides a new regulatory mechanism involved in lipid metabolism.
Cytosol-nucleus traffic of bile acids and subnuclear colocalization with FXR

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Introduction: Several nuclear receptors can be activated by bile acids (BAs), which have been suggested to reach the hepatocyte nucleus by translocation after binding to cytosolic proteins. Here we have investigated BAs cytosol-nucleus traffic and subnuclear colocalization with FXR.

Methods: Using freshly isolated rat hepatocyte nuclei uptake/efflux of fluorescein-tagged BAs (FBAs: CGamF, CDCGamF and UDCGamF) was measured by flow cytometry. Nuclear volume was modified by incubation in media containing varying sucrose concentrations (0–250 mM).

Results: A significant correlation between nuclear volume and load of FITC, FITC-dextran (4 kDa) or CGamF was found. The slope was FITC>FITC-dextran>>CGamF. Using confocal laser microscopy FBAs were located at the nuclear envelope and inside the nuclei both dispersed and concentrated in regions with less densely packed DNA, as suggested by propidium iodide staining. FXR was immunolocalized in these same regions. ATP affected nuclear accumulation and efflux of FBAs. To investigate the presence of export pumps in the nuclear envelope these membranes were isolated from hepatocytes nuclei and their purity tested by the abundance of nucleoporins p62, p152 and p90. Using Western blot analysis ABC transporters were detected in these membranes. Immunolocalization by confocal microscopy confirmed their presence at the nuclear envelope of rat hepatocytes.

Discussion/Conclusion: BAs can enter the hepatocyte nucleus in the absence of cytosolic proteins by partially selective pathways. The overall nuclear content is affected by the availability of aqueous space inside the nucleus, BAs binding to nuclear receptors and the activity of export pumps present at the nuclear envelope.
Intestinal cholesterol secretion is stimulated upon PPARδ activation

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Introduction: Traditionally, hepatobiliary cholesterol (CH) excretion is supposed to be the only way to excrete substantial amounts of CH from the body. However, we have shown with CH balance studies and intestinal perfusions with mice that a specific intestinal CH efflux pathway exists. Previous studies have shown that activation of PPARδ is associated with decreased CH absorption and increased fecal sterol secretion. In this study we investigated whether this activation not only affects intestinal absorption but also secretion.

Methods: For this purpose, we fed FVB wild type mice with the PPARδ agonist GW610742X with a daily intake of 20 mg/kg. After two weeks the mice were anaesthetized followed by bile duct cannulation to prevent bile entering the intestine. The proximal small intestine was then perfused for 90 min with buffer containing taurocholate/lecithin (10:2 mM) as CH acceptor.

Results: GW610742X had no effect on serum CH, nor was biliary CH secretion affected. In contrast, after GW610742X treatment intestinal CH secretion increased from 2.0 to 3.5 μmol/day.100 g, which can account for at least 60% of the twofold increase in neutral sterol output observed after PPARδ activation. Gene expression analysis of known genes involved in intestinal trafficking showed a change in NPC1L1. We therefore checked whether the NPC1L1 inhibitor ezetimibe influences intestinal CH secretion. No effect was observed.

Discussion/Conclusion: The previously observed increase in neutral sterol secretion induced by PPARδ activation can be explained in part by increased direct intestinal CH secretion. Clearly, this pathway is a prime target for therapies aiming at an increase in reverse CH transport.
Different regulation of ileal PPAR expression is responsible for increasing bile acid pool size in the rabbit but not rat after feeding cholesterol

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Introduction: We previously reported that the bile acid pool size expanded in rabbits fed cholesterol but not in rats and this difference caused their different responses of CYP7A1 to dietary cholesterol. We believe that increased expression of ileal apical sodium-dependent bile acid transporter (ASBT) in cholesterol-fed rabbits is responsible for the expansion of the bile acid pool size. Since human ASBT is up-regulated by PPAR, we studied whether PPAR is involved in the different regulation of ASBT in the rabbit and rat.

Methods: Rabbits and rats were fed 2% cholesterol (Ch) for 1 week. The ileal mucosa was collected from these animals for measurements of ASBT and PPARα mRNA levels by Northern blot and PPAR bound DNA (ASBT promoter) by chromatin immunoprecipitation assays (ChIP).

Results: ASBT and PPARα mRNA levels increased 1.7 and 2-fold respectively in the Ch-fed rabbits but did not change in rats. A functional PPAR binding element was identified in the rabbit ASBT promoter (-2490/-2457). In vitro study in Caco-2 cells demonstrated that PPARα increased the co-transfected rabbit ASBT promoter activity. The results from ChIP assays demonstrated that in the liver from Ch-fed rabbits, PPAR bound ASBT promoter increased as compared with those without Ch feeding whereas in Ch-fed rats, the PPAR bound DNA did not change.

Discussion/Conclusion: In rabbits but not rats, Ch feeding enhances PPAR expression which transactivates the ASBT promoter such that bile acid reabsorption is increased and the circulating bile acid pool size, expanded. We hypothesize that similar to the human, the rabbit but not the rat PPAR promoter contains an FXR binding element so that in Ch-fed rabbits where the intestinal bile acid increases, ileal FXR is activated and PPAR expression is induced.
The identification of bile acid regulated genes in oesophageal carcinogenesis – A comparative systems biology approach

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Introduction: Bile acids such as deoxycholic acid (DCA), present in gastro-oesophageal reflux disease (GORD), have been implicated in the development of Barrett’s oesophagus (BO) and subsequent progress to oesophageal adenocarcinoma (OAC). The transcriptional responses of two oesophageal cell lines, Het-1A (non-tumourogenic) and SKGT4 (cancer), to DCA, has been under investigation in our laboratory and through a genomic approach has identified cell type specific responses.

Methods: It is not as yet clear what part these DCA responsive genes may play in oesophageal carcinogenesis. Therefore, a novel informatic approach was taken to examine this question through integrating genomic studies of OAC and BO patient samples (Kimchi et al, GSE1420) with our genomic study of DCA signalling in oesophageal cells.

Results: Statistically significant gene lists were generated for both the BO/OAC study (2823 genes) and our experiment examining DCA mediated gene expression (HET-1A-2319 genes, SKGT4-1810 genes). These data sets were integrated through the use of statistical (GeneSpring) and system biology platforms (Metacore). These comparisons demonstrated that ~17.5% (493 genes) of genes altered in oesophageal carcinogenesis were responsive to DCA in HET-1A cells and ~15.9% (450 genes) DCA responsive in SKGT4s. Interestingly, unsupervised hierarchical clustering performed using these DCA responsive genes can distinguish between BO and cancer patient samples furthering their putative role in oesophageal carcinogenesis. Systems biology approaches generated gene networks and dissected gene lists by categories such as cell adhesion, death and movement.

Discussion/Conclusion: This study has for the first time attempted to elucidate the role of DCA in oesophageal carcinogenesis using a novel genomic and informatic approach.
Tribbles homolog 3 (TRB3) a novel regulator of bile acid signaling in oesophageal cells may be lost in oesophageal carcinogenesis

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Introduction: Deoxycholic acid (DCA) has been shown to be present in the reflux aspirates of GORD patients and has been postulated to be involved in oesophageal adenocarcinoma (OAC) promotion. Previous studies from our laboratory have demonstrated both increased levels of NF-κB activation and concomitant IL-8 expression in Barrett’s esophagus (BE) and EAC and their respective activation by bile acids.

Methods: Gene-expression-microarrays (Affymetrix) were utilised to examine DCA mediated gene induction in HET-1A-squamous and SKGT4-Tumour oesophageal cell lines. Gene induction was assessed using real-time RT-PCR (ABI) and TRB3 inhibition through siRNA-mediated knockdown (Dharmacon).

Results: The pseudo-kinase TRB3 was identified by transcriptomic screens of DCA signaling as a gene specifically induced in HET-1A cells, absent in the SKGT4 cancer cells and down-regulated cancer sequence microarray data. siRNA-mediated suppression of TRB3 in HET-1A cells resulted in increased levels of IL8 (5-fold) and IL6 (7-fold) over control cells. These findings are in keeping with the loss of TRB3 impacting on NF-κB/MAPK pathways leading to unchecked basal and inducible levels of cytokine gene expression as observed in BE/OAC and the cell line model. Additionally, DCA exposure of HET-1A cells resulted in the induction of CHOP expression (25-fold) which was reduced upon siTRB3 pre-treatment (12-fold) demonstrating a role for this protein in DCA mediated ER stress.

Discussion/Conclusion: Through a novel informatic and functional approach the relevance and genetic interaction of bile acid regulated genes such as TRB3 in esophageal carcinogenesis has been assessed. This study strengthens the role of secondary bile acids and such as DCA in OAC promotion.
Simple carbohydrate feeding decreases bile acid pool size and synthesis rate in C57Bl/6J mice

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The bile acid-activated nuclear receptor Fxr plays an important role in control of glucose homeostasis in addition to its established role in control of BA metabolism. Glucose has been shown to negatively regulate hepatic Fxr expression. We questioned whether a diet high in simple carbohydrates would affect bile acid metabolism in mice. Mice were fed a standard laboratory diet, high (40%) in complex carbohydrates, or a eucaloric semi-synthetic diet in which the carbohydrate fraction (40%) consisted solely of dextrose. Bile acid kinetics was assessed by stable isotope dilution. Bile acid pool size and cholic acid synthesis rate were significantly decreased (by 66% and 50%, respectively) in animals fed a dextrose-diet for 2 weeks as compared to chow-fed animals. Additionally, the fractional turnover rate was enhanced by 50%. Intestinal expression of Fxr was decreased whereas, interestingly, expression of the Fxr target gene Fgf15 was significantly enhanced (3 times) in dextrose-fed animals. Hepatic expression of Fxr remained unaffected. Coinciding with the low synthesis rate observed in dextrose-fed animals, hepatic expression of Shp was enhanced whereas expression of Cyp7a1 was decreased. Additionally, mice fed a dextrose-diet, developed hepatic steatosis: expression levels of lipogenic genes were clearly enhanced as compared to chow-fed animals. We conclude that a diet high in simple carbohydrates has significant effects on bile acid metabolism in mice: altered bile acid metabolism might contribute to adaptations in glucose and fat metabolism.
In vitro associations of unconjugated bilirubin with fat and bile salt

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Introduction: Unconjugated hyperbilirubinemia, such as found in neonatal jaundice or in Crigler Najjar disease, is usually treated with phototherapy. Previously, we demonstrated that the lipase inhibitor orlistat (Orl) decreased plasma unconjugated bilirubin (UCB) levels and simultaneously stimulated fecal fat excretion. These results suggested that Orl induces intraluminal UCB capture through hydrophobic association with intraluminal fat, preventing its transmucosal reabsorption. We now evaluated in vitro the interactions between fat, UCB and bile salts in an aqueous environment.

Methods: Solutions of taurocholate (TC, 10–20 mM) and UCB (final concentration 10 µM) were incubated with linoleic acid (LFA; 0–10 mM UCB, pH 7.4, 37°C, 15 min) after which samples were centrifuged. UCB was then quantified in supernatant and precipitate (absorption spectrophotometry, HPLC).

Results: UCB concentration in supernatant decreased in a dose-dependent fashion with simultaneous appearance in the precipitate upon addition of LFA to the incubation mixture. This was prevented by increasing the TC concentration in the supernatant. Maximal decrease from the supernatant at 10 mM LFA: TC 10 mM, -71% ± 2%; TC 15 mM, -55% ± 7%; TC 20 mM, -20% ± 13% (each p < 0.01). UCB in supernatant was negatively correlated to LFA concentration at all TC concentrations (r = -0.66, p < 0.001).

Discussion/Conclusion: Present results indicate that LFA competes with UCB for incorporation into bile salt micelles, thereby decreasing the amount of solubilized UCB. These in vitro results support the concept that fat malabsorption enhances the disposal of UCB from the intestine via the feces by decreasing its solubilization, and not by direct association of UCB to unabsorbed fat.
High fat diet-induced FGF19 resistance in livers of C57bl6 mice

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Introduction: Activation of ileal FXR by bile salts induces release of fibroblast growth factor 19 (FGF19) in the portal circulation with subsequent repression of hepatic Cyp7a1 mRNA via a pathway involving FGFR4. FGF19 has also been implicated in lipid homeostasis and control of small intestinal immune response. We hypothesize that FXR/FGF19 signaling may be disturbed in NAFLD/NASH. We evaluated the effect of mild expression of FGF19 in chow-fed and high-fat diet (HFD) fed, i.e. steatotic mice.

Methods: Chow-fed and HFD-fed mice were injected with mock- or FGF19-adenovirus. Four days after injection, mice were sacrificed and livers were studied by histochemistry. Plasma FGF19 levels were determined by ELISA, and hepatic transcript levels were determined by quantitative RTPCR.

Results: In chow-fed mice, the expression of both Cyp7a1 and Cyp8b1 mRNA are downregulated by FGF19. Interestingly, levels of mRNAs encoding fatty acid synthase (FAS) and acetyl-CoA carboxylase 2 (ACC2) were significantly lowered by FGF19 (-50%, [p = 0.001] and -40%, [p = 0.049], respectively), suggesting that FGF19 may affect liver lipid synthesis and fatty acid oxidation. HFD-fed mice developed a fatty liver. Administration of FGF19-adenovirus to these steatotic mice did not significantly downregulate the expression of either Cyp7a1 mRNA or Cyp8b1 mRNA, although a negative trend was observed. FAS mRNA expression remained downregulated (-40%, p = 0.024) by FGF19 in steatotic mice, while ACC2 mRNA levels in FGF19-treated animals were comparable to controls.

Discussion/Conclusion: In conclusion, HFD-fed mice appear less responsive to the suppressive effects of FGF19 on studied transcript levels (Cyp7a1, Cyp8b1, ACC2), suggesting an FGF19-resistant state in these animals.
Downexpression of intestinal Niemann-Pick C1 like 1 protein (NPC1L1) by ezetimibe prevents diet-induced fatty liver diseases in mice

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Introduction: Deletion of the liver X receptor alpha (LXRalpha) gene results in severe lipid accumulation in the livers of mice challenged to a high fat diet because of dysfunctional lipid metabolism in the liver (Cell, 1998; 93: 693), suggesting that LXRalpha (-/-) mice are an excellent model of diet-induced fatty liver diseases. The Western diet containing high cholesterol and high fat is an important risk factor for fatty liver diseases. We observed recently that NPC1L1 has a crucial effect on the regulation of intestinal absorption of not only cholesterol but also fatty acid.

Aims: We explored whether ezetimibe inhibits intestinal absorption of both fatty acid and cholesterol, and in turn, prevents diet-induced fatty liver diseases in mice.

Methods: Male LXRalpha (-/-) mice (n = 3–5 per group) were fed a rodent chow diet, or a special diet (15% butterfat, 1% cholesterol and 0.5% cholic acid) supplemented with ezetimibe at 0 or 200 micrograms/day for 56 days. Intestinal absorption of fatty acid and cholesterol was determined by balance methods. Plasma and liver lipids were measured by biochemical methods. Liver histology with H&E and Oil red O staining was studied.

Results: On chow, feeding ezetimibe reduced intestinal absorption of fatty acid from 86 ± 9% to 35 ± 6%, and of cholesterol from 50 ± 6% to 5 ± 1%, respectively. Under the special diet conditions, plasma concentrations of triglyceride and cholesterol were significantly decreased by ezetimibe, from 162 ± 15 mg/dl to 72 ± 8 mg/dl and from 118 ± 9 mg/dl to 80 ± 6 mg/dl, respectively. Morphological and histological studies of liver found that the number and size of intracellular vacuoles (characteristic of lipid deposits) was greater, and neutral lipid staining of liver sections verified the deposition of increasing quantities of lipid in mice fed the special diet and receiving no ezetimibe, compared with chow-fed mice. However, treatment of ezetimibe completely prevented lipid accumulation in the liver, even under the special diet conditions. Furthermore, hepatic total triglyceride (14 ± 3 mg/g liver tissue) and cholesterol (10 ± 2 mg/g liver tissue) contents were significantly lower in ezetimibe-treated mice, compared with mice fed the special diet and receiving no ezetimibe (75 ± 10 mg/g liver tissue and 39 ± 6 mg/g liver tissue, respectively).

Discussion/Conclusion: Ezetimibe can prevent fatty liver diseases in mice challenged to a high fat and high cholesterol diet by inhibiting intestinal absorption of both fatty acid and cholesterol through the NPC1L1 pathway.
The enterohepatic circulation of bile salts in mice in vivo: Effects of essential fatty acid (EFA) deficiency

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Introduction: Essential fatty acid (EFA) deficiency is a frequent complication of fat malabsorption, for example during cholestasis or in cystic fibrosis patients. In mice, EFA deficiency induces fat malabsorption despite increased bile production. It has remained unclear, to what extent EFA deficiency affects bile salt homeostasis. We aimed to determine the effects of EFA deficiency on synthesis and enterohepatic circulation of cholate, the major bile salt species in mice.

Methods: After 8 weeks of control or EFA-deficient diet, we determined the biochemical marker for EFA deficiency, the triene/tetraene ratio, and different parameters of the enterohepatic circulation of cholate in vivo by stable isotope methodology in FVB mice. The bile salt pool composition was determined by gas chromatography and hepatic and intestinal mRNA expression by Q-PCR.

Results: Mice fed the EFA-deficient diet had a triene/tetraene ratio of 0.23 ± 0.06 vs. 0.01 ± 0.00 in control mice (p < 0.001). EFA-deficient mice had increased cholate synthesis rate (+29%, p < 0.05), pool size (+59%, p < 0.05), and intestinal reabsorption (512 ± 275 vs. controls, 156 ± 29 µmol/100 g/day, p < 0.05). The fraction of cholate that was not reabsorbed per enterohepatic cycle was lower in EFA-deficient mice (2.6 ± 1.6% vs. 4.8 ± 1.6% in controls, p < 0.05). EFA deficiency did not affect biliary bile salt composition or hepatic mRNA expression of Cyp7a1, Bsep, Ntcp, FGFR4 or FXR. Interestingly, EFA deficiency was associated with reduced mRNA expression of Fgf15 in the terminal ileum (p < 0.05).

Discussion/Conclusion: EFA deficiency in mice increases the synthesis rate of cholate and its conservation in the enterohepatic circulation.

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Effect of FXR deficiency on fed and fasted energy expenditure

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Introduction: The farnesoid X receptor (FXR) is a nuclear receptor that is heavily expressed in enterohepatic tissues, where it acts to maintain bile salt homeostasis. Actions of FXR extend to regulate glucose and lipid metabolism. Bile acids, the natural ligands of FXR have shown to increase energy expenditure. However precise mechanisms and the role of FXR herein are not understood. To investigate FXR’s role herein, we utilized the doubly labeled water technique (DLW) to measure daily energy expenditure (DEE) in unrestrained fed and fasted Fxr wildtype (FXRWT) and Fxr deficient (FXRKO) mice, the latter being a mouse model displaying elevated plasma bile salt levels.

Methods: 5 FXRWT and 5 FXRKO (male, 11 wks old, on standard chow) were injected about 0.1 g DLW. After an equilibration period of 1h, an initial blood sample was obtained via tail puncture. And 24 h later the fed blood sample was obtained. Thereafter, food was removed and the fasted blood sample was collected another 24 h later. Rate of CO₂ production was calculated from fractional turnover rates of ²H and ¹⁸O.

Results: No differences were observed between FXRWT and FXRKO animals in both the fed and fasted states for absolute and mass-specific DEE (1.95 ± 0.14 vs. 1.89 ± 0.14 KJ/g BW fed; 1.59 ± 0.5 vs. 1.62 ± 0.23 KJ/g BW fasted), total body water volume and turnover rates.

Discussion/Conclusion: Fed normal chow, FXR deficiency did not alter DEE in fed or fasted state. A high fat dependent positive effect of bile salts on DEE has been reported. The effect of Fxr herein needs to be explored.
Side-chain modification of ursodeoxycholic acid alters cellular hyper-proliferation and lipid homeostasis in a mouse model of cholestatic liver injury


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Introduction: Mdr2 knockout mice (Mdr2-/-) develop cholestatic liver injury, characterized by cellular hyper-proliferation, liver fibrosis and cancer. Although being unable to secrete phospholipids (PL) and cholesterol (CH) into bile, these mice do not accumulate hepatic CH/PL and even more interestingly show reduced amounts of serum CH/PL. 24-norursodeoxycholic acid (norUDCA) treatment in Mdr2-/- mice completely reverses liver injury and abrogates cellular hyper-proliferation and liver fibrosis. However, the exact molecular mechanisms underlying norUDCA action remain unclear.

Aims: to (i) identify molecular targets leading to disturbed lipid and cell cycle homeostasis; (ii) unravel potential therapeutic mechanisms of norUDCA action.

Methods: Microarray analysis was performed from livers of wildtype and Mdr2-/- mice either receiving standard chow or supplemented with norUDCA. Target genes were validated by Q-PCR, Western blot and Immunohistochemical analysis. Hepatic and serum lipid composition were assessed by biochemical analyses, GC/MS and ESI-MS/MS.

Results: Expression of several target genes triggering cell cycle progression and proliferation including c-Jun, cyclin D/E were significantly up-regulated in Mdr2-/- and reversed to normal levels by norUDCA treatment. Moreover, norUDCA blocked selectively activation of mTOR, p70s6k and Rps6. Interestingly, other bile acids (CA, UDCA) – instead of blocking – directly activated this signal transduction cascade. Besides recovery of liver function, norUDCA restored serum CH/PL-levels in Mdr2-/- mice and was found to modulate key components of the CH/PL biosynthesis pathway in both genotypes.

Discussion/Conclusion: Our data provide novel mechanistic/metabolic insights into the role of Mdr2 in chronic liver injury and demonstrate the effectiveness of norUDCA to modulate lipid metabolism and cell cycle homeostasis.
The hydrophobic iminosugar AMP-DNM attenuates hepatic steatosis and increases reverse cholesterol transport in ob/ob mice

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Introduction: Glycosphingolipids have been shown to influence insulin signalling. For instance, mice deficient in GM3 synthase are resistant to high fat diet induced insulin resistance.

The aim of this study was to investigate the influence of modulation of glycosphingolipid synthesis on hepatic steatosis and lipid homeostasis in leptin deficient ob/ob mice.

Methods: Leptin-deficient (ob/ob) mice were fed standard lab chow with or without 25 mg/kg/day AMP-DNM (N-(5'-adamantane-1'-yl-methoxy)-pentyl-1-deoxynojirimycin). After four weeks of treatment the bile duct of these mice was cannulated and bile was collected for 15 minutes. Liver and blood were harvested. Stools were collected for assessment of faecal neutral sterol excretion.

Results: AMP-DNM was well tolerated and there was no difference in growth between control and treated mice. Four weeks of dietary AMP-DNM decreased plasma glucose levels 22 ± 6 to 12 ± 4 mmol/L and decreased liver glucosylceramide and ganglioside GM3 concentrations by about 30% and 45% respectively. AMP-DNM induced a ±25% decrease in liver triglyceride, but had no effect on liver cholesterol and phospholipids. Biliary secretion of bile salt, phospholipids and cholesterol was unaltered by the treatment but plasma cholesterol increased by ±20% and phospholipids and triglycerides were unaltered. Interestingly faecal neutral sterol excretion was two-fold higher in AMP-DNM treated animals.

Discussion/Conclusion: Four weeks treatment of ob/ob mice with the hydrophobic iminosugar AMP-DNM ameliorates many symptoms of the metabolic syndrome in these mice including plasma glucose levels and hepatic steatosis. The increase in faecal neutral sterol secretion suggests that AMP-DNM might stimulate reverse cholesterol transport.
Pregnancy-induced liver growth: A role for bile acids?

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Introduction: Bile acids (BA) stimulate liver growth and rise during gestation. Metabolic requirements of the maternal liver increase during pregnancy and we propose that this is achieved by liver growth triggered by raised circulating BA. We aimed to investigate whether pregnancy-induced liver growth is analogous to bile acid-induced growth in mice.

Methods: Physiological measurements, hepatic microarrays and Ki-67 staining were performed on pregnant (18-days-post-conception) animals, on mice fed a 0.5%-cholate (CA) diet and on non-pregnant animals fed a control diet.

Results: Serum bile acids were raised by CA feeding (14-fold, p < 0.01) and pregnancy (7-fold, p = 0.056) and an increase in liver size was induced by CA feeding (50%, p < 0.01) and pregnancy (65%, p < 0.01), indicating a possible link between bile acid-induced and pregnancy-induced liver growth. CA feeding and pregnancy increased the number of proliferating hepatocytes (13.5-fold, p < 0.01 and 6-fold, p < 0.05) but pregnancy also increased hepatocyte size (18.5%, p < 0.05) whereas CA feeding had no effect. Preliminary hepatic microarray analysis revealed that genes involved in hepatic hypertrophy are differentially expressed by pregnancy (p < 0.01) but not by CA-feeding, indicating that the mechanisms of liver growth may overlap but are not identical.

Discussion/Conclusion: Pregnancy increases serum BA concentrations and liver size. However, liver growth caused by BA feeding is a result of hyperplasia alone, while pregnancy-induced growth is a result of both hyperplasia and hypertrophy. These data indicate that BA are not the sole inducer of liver growth in pregnant mice.
Bile acid binding resins improve metabolic control through the induction of energy expenditure

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Introduction: Over the 5 years, the field of bile acid (BA) research has undergone a considerable evolution. Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, it has recently become clear that BAs are also biological signaling molecules. We have shown that BA decreased hepatic VLDL production via FXR pathway (Watanabe et al, JCI, 2004) and increased energy expenditure, preventing obesity and insulin resistance (Watanabe et al, Nature, 2006). These observations build a strong case that BA has effects beyond the strict control of function as metabolic integrators.

Methods: We evaluated the metabolic effect of BA binding resins (BABRs) by administrating either colestimide or cholestyramine to both diet-induced obesity model and genetic model of metabolic syndrome. Furthermore, to test the clinical efficacy of BABR in humans with metabolic syndrome, a small open-label clinical trial in hypercholesterolemic patients with overweight was initiated.

Results: Administration of BABRs increased energy expenditure, translating into significant weight reduction and insulin sensitisation. This metabolic effect of BABRs coincides with activation of BA synthesis in liver and thermogenesis in BAT. These effects of BABR occur despite normal food intake and triglyceride absorption. Also in humans, BABRs induce significant weight loss resulting in a marked insulin sensitisation. BABR and BA had similar effects on BA composition and thermogenesis viaTGR5 activation.

Discussion/Conclusion: Our data hence suggest that BABRs could be useful for the management of the metabolic syndrome, since they not only lower cholesterol levels, but also reduce obesity and improve insulin resistance.
Curcumin improves liver injury in Mdr2 (Abcb4) knockout mice by inducing anti-oxidative response, but not via modification of bile acid transport/metabolism

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Introduction: Sclerosing cholangitis in multidrug resistance protein 2 (Abcb4) deficient (Mdr2-/-) mice results from bile acid toxicity due to absent phospholipid excretion into bile. Curcumin was shown to attenuate hepatic damage in several animal models of liver injury. However, its influence on bile acid metabolism has not been explored. We studied influence of curcumin on liver injury and bile acid transport and metabolism in Mdr2-/- mice.

Methods: Mdr2-/- mice received curcumin-enriched diet for 4 weeks. Serum liver tests, liver morphology, markers of inflammation, cell proliferation, fibrosis, bile formation and key hepatobiliary transporters were studied.

Results: Compared to untreated controls curcumin feeding decreased liver damage (ALT: 384 ± 43 vs. 173 ± 28 U/L, ALP: 338 ± 35 vs. 241 ± 13 U/L, bile acids: 58 ± 11 vs. 30 ± 2 μM/L; p < 0.02) and fibrosis (hepatic hydroxyproline content: 166 ± 2 vs. 130 ± 8 mg/g. liver, Col1a2 expression: 2-fold reduction; p < 0.05). Both hepatocellular and cholangiocellular proliferation as well as cholangiocyte activation marker VCAM-1 decreased by curcumin. Additionally, curcumin increased bile flow (1.2 ± 0.2 vs. 1.8 ± 0.2 μL/min/g. liver; p < 0.02) and biliary glutathione content (1 ± 0.3 vs. 2.2 ± 1.3 μM/μL; p < 0.02). However, neither bile acid synthesis (Cyp7a1), nor key hepatocellular transporters (Ntcp, Mrp2, Mrp3, Mrp4, Bsep) were altered.

Discussion/Conclusion: Curcumin improves liver injury in sclerosing cholangitis by modulating anti-oxidative responses but not bile acid homeostasis.
Bile acid inhibition of human hepatic stellate cell proliferation is COX-2 mediated and requires the combined and sequential activation of PKCα, p38 MAPK and FXR

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Introduction: We have previously shown that the bile acid chenodeoxycholate (CDCA) inhibited the proliferation of HSC through at least an increased cell cycle arrest in G0/G1 phase and that this effect was cyclooxygenase (COX)-2 dependent. The present aim was to study, at the molecular level, the mechanism regulating COX-2 protein expression by bile acids.

Methods: HSC isolated from human liver biopsies by an outgrowth method were cultured using standard conditions. Isolated HSC were characterized immunohistochemically using specific antibodies against αSMA and desmin and over 98% of cells were positive for these proteins. Protein expression and mRNA was measured by Western blotting and RT-PCR, respectively.

Results: CDCA stimulated COX-2 mRNA and protein expression in a time- and dose-dependent manner with a > 11-fold increase with 100 μM CDCA. This CDCA-induced increased COX-2 expression was >70% abolished by preincubating the cells with either Gö6976 (cPKC inhibitor), SB203580 (p38 inhibitor) or guggulsterone (FXR inhibitor). Similar findings were generated when the respective dominant negative (DN) mutant was used. In addition, both Gö6976 and PKCα DN mutant prevent the phosphorylation of p38. Incubation of the cells with CDCA resulted in 5-10 fold increase in FXR protein expression, which was blocked at least by SB203580 preincubation. Taken together, these data indicate that CDCA increases COX-2 protein expression via a PKCα-mediated, FXR-facilitated pathway, in which p38 is downstream of PKC and upstream of FXR.

Discussion/Conclusion: This study underlines the role of PKCα, p38 MAPK and FXR as antifibrotic targets in liver injury and fibrogenesis.
Glycoursodeoxycholic acid prevents the cascade of neurodegeneration events caused by bilirubin

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In previous studies we demonstrated that glycoursodeoxycholic acid (GUDCA) prevents the immunostimulating effects of unconjugated bilirubin (UCB) on astrocytes. This study aimed to investigate whether GUDCA is able to abrogate UCB-induced cell death, as well as impairment of energy metabolism and of neurite development in immature neurons.

Rat neurons at 3 days in vitro (DIV) were exposed to 50 µM UCB and 100 µM human serum albumin, for 1 h at 37º C to evaluate energy status (mitochondrial depolarization by TMRE staining and the metabolites of glycolysis by enzymatic assays) and cell death by apoptosis (flow cytometric analysis). To assess injury to neurite outgrowth cells were incubated with UCB during 24 h. UCB was removed and cells additionally cultured until 18 DIV. Neurites were labelled with anti-MAP2 antibody, and their extension and number of nodes were determined using ImageJ software. Neuroprotective effects of GUDCA were determined in cells treated for 1 h with 50 µM GUDCA prior to UCB addition.

GUDCA was able to prevent UCB-induced apoptosis (~67%, p < 0.05), mitochondrial dysfunction (~56%, p < 0.05) and alterations of glycolytic metabolism (~100% in fructose 1,6-bisphosphate/fructose 6-phosphate and ~86% in intracellular lactate; p < 0.01). In addition, GUDCA counteracted the long-term effects of UCB on neuritic network (~100%, p < 0.05).

These data show that GUDCA prevents apoptosis, mitochondrial depolarization and up-regulation of glycolysis resulting from a short exposure to UCB, as well as the delayed effects on neurite disassembly. Therefore, GUDCA appears as a new therapeutic approach to prevent neuronal damage and neurodevelopmental abnormalities by neonatal hyperbilirubinemia.

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Hepatotoxicity associated with unconjugated and conjugated bilirubin species, and beneficial impact of ursodeoxycholic and glycuroursodeoxycholic acid

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Elevation of unconjugated and conjugated bilirubin species is common in several liver diseases and may cause cholestasis, although the underlying mechanisms remain elusive. Ursodeoxycholic acid (UDCA) is used in the therapy of cholestatic liver diseases, but its efficacy on protecting hepatocytes from bilirubin-induced toxicity is unknown.

Hence, the purpose of this study was to evaluate the toxic effects of a combination of unconjugated bilirubin (UCB) and conjugated bilirubin (CB) on the loss of viability and caspase activation in HepG2 cells, and the ability of either UDCA or its glycine conjugated species (GUDCA) to prevent bilirubin injury.

HepG2 cells were treated with 50 μM UCB + 50 μM CB (ditaurine amide) in the presence of 100 μM of human serum albumin for 72 h. In parallel experiments, cells were treated with 30 μM UDCA or 30 μM GUDCA within the last 24 h of incubation. Caspase-3, -8 and -9 activities were assessed by using specific substrates and cytotoxicity by the MTS reduction assay.

Exposure of HepG2 to UCB + CB produced decreasing cell functionality (~0.6-fold), and increasing caspase-3 (~6.0-folds), caspase-8 (~7.0-folds) and caspase-9 (~7.5-folds) activities. Interestingly, both UDCA and GUDCA treatments were able to prevent the activation of caspase-3 (UDCA: ~40%; GUDCA: ~30%), caspase-8 (UDCA: ~30%; GUDCA: ~40%), and caspase-9 (UDCA: ~10%; GUDCA: ~15%), and the loss of cell function (UDCA: ~10%; GUDCA: ~20%).

Collectively, these results provide an insight into possible routes of bilirubin-induced hepatotoxicity and suggest that both UDCA and GUDCA may be promising therapeutic agents for cholestatic jaundice.

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Reversal of bilirubin-induced changes in neuronal redox status by glycoursodeoxycholic acid

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Neuronal damage by unconjugated bilirubin (UCB) appears to be mediated by oxidative stress. Although ursodeoxycholic acid (UDCA) has been reported as a cytoprotective and antioxidant molecule, there are no reports on the preventive effects of its glycine-conjugated derivative (GUDCA) in oxidative damage to nerve cells. Here, we assessed the redox status of neurons exposed to UCB and evaluated the ability of GUDCA to abrogate the oxidative damage. Cultured rat neurons were incubated with 50 or 100 μM UCB in the presence of 100 μM human serum albumin, for 4 h at 37° C. In parallel experiments cells were pre-treated for 1 h with 50 μM GUDCA. Protein carbonyls, 4-hydroxy-2-nonenal-protein adducts, intracellular glutathione (GSH) content and cell death were determined. The results obtained showed that GUDCA counteracts the UCB-induced oxidative injury by more than 20% (P<0.05), thus restoring the basal status. GUDCA was also able to hinder the ~20% decrease of GSH originated by UCB (p < 0.05), indicating that the GUDCA antioxidant defense mechanism is mediated by glutathione. Furthermore, GUDCA prevented the UCB-induced release of LDH by ~78% (p < 0.01), demonstrating its ability to protect neurons demise. Collectively, these data show that oxidative stress is one of the pathways associated with neuronal viability impairment by UCB, and that GUDCA significantly prevents such effects from occurring. This observation warrants further investigation to determine the utility of prophylactic GUDCA in preventing UCB-induced neurotoxicity due to oxidative stress.

Altered bile composition after human liver transplantation: A contributing factor for development of non-anastomotic biliary strictures

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Introduction: Non-anastomotic strictures (NAS) are considered to be the most troublesome biliary complication after liver transplantation. Although the pathogenesis of NAS is not completely clear, experimental studies have suggested that bile salt toxicity is involved. Aim of this study was to identify the role of bile salts in the development of NAS after clinical transplantation.

Methods: One-hundred-eleven adult liver transplant recipients were prospectively studied. Bile samples were collected daily during the first 8 days after transplantation to determine biliary concentrations of bile salts, phospholipids, and cholesterol. Perioperative changes in the hepatic expression of bile transporters were assessed using real time RT-PCR.

Results: NAS was detected in 14/111 patients (12.6%) within one year after transplantation. Median time between transplantation and detection of NAS was 2.4 months. There were no significant differences in patient and donor characteristics between patients who developed NAS and those who did not. Postoperative serum liver enzymes were also similar in both groups. The biliary secretion of bile salts, phospholipids and cholesterol, however, was significantly lower in patients who developed NAS, compared to patients who did not. Simultaneously, the bile salt:phospholipid ratio was significantly higher in the patients developing NAS. Hepatic expression of bile salt transporters increased after transplantation, but there were no significant differences between the groups.

Discussion/Conclusion: Although early after transplantation patients developing NAS are clinically indiscernible from patients who do not develop NAS, there are pronounced differences in bile composition. Patients who will develop NAS have a significantly lower bile salt and phospholipid secretion, and a higher biliary bile salt:phospholipid ratio during the early postoperative period. These findings suggest that bile salts are involved in the pathogenesis of NAS.
Opposing effects of deoxycholic acid and ursodeoxycholic acid on Golgi structure; implications for colon cancer progression

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Introduction: Deoxycholic acid (DCA) is a hydrophobic bile acid implicated as a tumour promoter in colon carcinoma. We observed DCA caused Golgi fragmentation, an organelle responsible for protein processing in the cell. On the other hand ursodeoxycholic acid (UDCA), a hydrophillic bile acid prevents DCA-induced Golgi fragmentation.

Aims and background: This study was undertaken to investigate the mechanisms of DCA-induced Golgi fragmentation and determine how UDCA pre-treatment can reverse this process.

Method: HCT116 colon carcinoma cells were treated with DCA or UDCA and Golgi were visualised by immunofluorescence using a GM130 antibody. Fragmentation was assessed by high throughput screening and quantified using the Incell analyser 1000. Over-expression and siRNA were used to investigate mechanisms involved in the fragmentation process.

Results: DCA fragmented the Golgi in HCT116 cells. Pre-treatment with UDCA prevented DCA-induced Golgi fragmentation. The synthetic glucocorticoid dexamethasone could also prevent DCA-induced fragmentation. The Golgi packages and transports proteins by a process called membrane fission which involves PKC\(\eta\) and PKD. HCT116 cells transfected with constitutively active (CA)-PKC\(\eta\) had fragmented Golgi, whereas pre-treatment with UDCA or dexamethasone prevented CA-PKC\(\eta\)-induced fragmentation. DCA activated PKD, whereas UDCA or dexamethasone pre-treatment prevented DCA-activation of PKD. UDCA inhibition of DCA-induced Golgi fragmentation was reversed by siRNA knockdown of the glucocorticoid receptor (GR).

Conclusion: DCA-induced fragmentation may be due to overactivation of the membrane fission process via activation of PKC\(\eta\) and phosphorylation of PKD. These effects were reversed by UDCA which acts through the GR. This study identifies DCA as the first physiological inducer of Golgi fragmentation, and suggests UDCA may be used as a chemopreventative agent. This study also gives an insight into mechanisms of bile-acid signalling which may be involved in neoplastic progression in the colon.
Glutathione and hydrogen peroxide detoxifying enzymes are important in the protection of hepatic stellate cells against oxidative stress

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Introduction: In many chronic liver diseases, including chronic cholestasis, the hepatic stellate cells (HSC) are exposed to elevated levels of reactive oxygen species and bile acids. Since HSC proliferate in chronic liver diseases, they must be well protected against reactive oxygen species and oxidative stress. The aim of this study is to evaluate the importance of anti-oxidants and ROS-scavenging enzymes in the protection against oxidative stress-induced

Methods: Serum-starved culture-activated rat HSCs were exposed to oxidative stress induced by hydrogen peroxide (0.2–5 mM). Apoptosis and necrosis were determined by Acridine Orange and Sytox Green/Hoechst 33342 nuclear staining, respectively. The hydrogen peroxide scavenging enzymes catalase and glutathione-peroxidase (GPx) were inhibited using 3-aminotriazole and mercaptosuccinic acid, respectively. The anti-oxidant glutathione was depleted by treatment with bithionine sulfoximine (BSO) at 200 μM and repleted with the cell permeable GSH-analogue GSH-monoethylester (GSH-MEE).

Results: Hydrogen peroxide did not induce cell death at concentrations up to 1 mM hydrogen peroxide, but induced > 90% necrosis at concentrations of 5 mM. However, glutathione depletion dramatically increased the sensitivity of HSCs to hydrogen peroxide, resulting in > 70% necrosis at 0.2 mM hydrogen peroxide. This sensitizing effect was abolished by the GSH-analogue GSH-MEE. Blocking catalase or GPx did not sensitize HSCs to hydrogen peroxide induced necrosis, but greatly increased sensitivity of HSCs to hydrogen peroxide induced apoptosis.

Discussion/Conclusion: To resist oxidative stress, HSC are dependent on anti-oxidants and ROS-scavenging enzymes. GSH is important to prevent ROS-induced necrosis, whereas catalase and Gpx are important to prevent ROS-induced apoptosis.
Immature cardiomyocytes are more susceptible to bile acid-induced arrhythmia in embryonic stem cell-derived cardiomyocyte models

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) is associated with maternal liver impairment, raised serum bile acids, premature delivery and intrauterine death. We have previously demonstrated that bile acid-induced arrhythmias in cardiomyocytes are calcium related and hypothesised that this is the cause of the fetal death. While fetal arrhythmias are reported in ICP, maternal arrhythmias have not been demonstrated. We used two in vitro models of the fetal heart; mouse embryonic stem cell-derived cardiomyocytes (mESC-CM) and human (hESC-CM) to investigate whether susceptibility to the arrhythmogenic effect of the bile acid taurocholate (TC) (0.1–1.0 mM) alters with cardiomyocyte maturation.

Methods: We used scanning ion conductance microscopy to measure contraction in both models.

Results: Addition of 0.1 mM TC to mESC-CM at an early stage of differentiation caused asynchronous beating, 55% reduction of amplitude of contraction (SEM = 0.67; p < 0.001) and was more marked at higher TC concentrations with a non-reversible 65% reduction of amplitude (SEM = 0.66; p < 0.001). At the terminal stage of differentiation TC had non-significant effects on contraction. TC-induced intracellular calcium release was associated with desynchronisation of mESC-CM networks. In hESC-CM at < 30 days of differentiation, 0.1 and 1.0 mM TC reduced contraction amplitude by 20% (SEM = 2.6; p < 0.001) and 42% (SEM = 2.3; p < 0.001) respectively, and this was irreversible. More mature hESC-CM (> 30 days) were not affected by either concentration.

Discussion/Conclusion: These findings show that susceptibility to bile acid-induced arrhythmias decreases with maturation in culture for both mESC-CM or hESC-CM. We propose that these models will be of value in the investigation of maternal and fetal arrhythmia.
Colitis and sclerosing cholangitis: Intestinal inflammation-associated changes of bile secretion and hepatobiliary transporter expression in mice

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Background and aims: The pathogenetic link between ulcerative colitis and primary sclerosing cholangitis (PSC) remains poorly understood. Translocation of lipopolysaccharide (LPS) or other bacterial products could contribute to cholestasis via inflammation-induced changes in hepatobiliary transporter gene expression. We therefore examined the effects of acute colitis on bile secretion and expression of proinflammatory mediators, hepatobiliary transporters and their regulatory nuclear receptors in mice. Furthermore, we tested the potential role of chronic colitis as "second hit" in heterozygous multidrug resistance gene 2 knockout mice (Mdr2 +/-) which contrary to homozygotes (Mdr2-/-) do not spontaneously develop SC.

Methods: Acute colitis was induced in C57/BL6 mice with 3% dextran sulfate sodium (DSS) for 7 days. Chronic colitis in Mdr2+/- and wildtype Mdr2+/+ was induced by 5 cycles of DSS. In acute colitis, bile flow/composition and hepatic mRNA expression (RT-PCR) was compared with LPS treated animals (15 mg/kg BW i.p.). The impact of chronic colitis on bile formation and liver histology was compared between Mdr2+/+ and +/- animals.

Results: Acute DSS colitis increased biliary output of bile acids (BA; 210%, p < 0.05) and phospholipids (PL; 210%, p < 0.05) but not of cholesterol (113%), glutathione (95%) and bile flow (114%, n.s.). Mdr1a (1.7-fold, p < 0.05) and Ostα (1.8-fold, p < 0.05) mRNA was induced, while other transporters (Ntcp, Bsep, Mrp2- 4, Ostβ, Abcg5, Abcg8, Oatp1- 4, Mdr1b, Mdr2), nuclear receptors (RXRα, FXR, CAR, PXR) or proinflammatory mediators (TNFα, iNOS) remained unchanged. In comparison, LPS reduced Ntcp (29%), Bsep (22%), Mrp2 (52%) and induced Mdr1b (1.7-fold), TNFα (6.8-fold) and iNOS (12-fold; all p < 0.05). BA/PL ratios did not change in acute (7.84 vs. 8.68 in controls) and chronic DSS colitis (10.27 vs. 7.73) but decreased after LPS (2.19). Chronic colitis induced mild portal inflammation in Mdr2+/- but not Mdr2+/+. However, the full histological picture of SC was not observed.

Conclusions: LPS but not acute DSS colitis reduced hepatobiliary gene expression and bile secretion. Although chronic colitis in Mdr2+/- mice led to mild portal inflammation, full-blown SC was not induced. These findings suggest that intestinal inflammation-associated changes of hepatobiliary transporter expression may not represent a major pathogenetic factor for development of SC.
Collagenous colitis is associated with genetic variants of FXR and MDR3 along with high-producer TNFα genotypes

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Introduction: Collagenous colitis is a condition of unknown aetiology characterised by watery diarrhoeas and histological deposition of a sub-epithelial collagen in the colonic mucosa. Characteristically, there is evidence for bile acid malabsorption and there is also an increased frequency of systemic autoimmunity among the patients. We investigated a large panel of polymorphisms in genes encoding proteins involved in bile acid homeostasis as well as pro-inflammatory cytokines in a well-defined cohort of collagenous colitis patients.

Methods: Ninety-one German collagenous colitis patients and 1096 healthy blood donors were genotyped for polymorphisms in 22 genes relevant to the collagenous colitis phenotype using SNPlex® technology (Applied Biosystems, Foster City, USA). Statistical analyses were performed using SPSS® (SPSS Inc., IL, USA) and Haploview v3.2 (http://www.broad.mit.edu/mpg/haploview/).

Results: Associations with collagenous colitis robust to correction for multiple testing by gene-wise permutations were found at the coding (Gly652Arg) single nucleotide polymorphism (SNP) rs8187799 in the multidrug resistance 3 (MDR3, ABCB4) gene (\(\text{OR}_{\text{minor allele (min)}} = 1.8, 95\% \text{ CI} [1.1–3.1], p_{\text{corrected}} = 0.01\)), the intronic rs1030454 SNP in the farnesoid X receptor (FXR) gene (\(\text{OR}_{\text{min}} = 1.9, 95\% \text{ CI} [1.2–2.9], p_c = 0.04\)), and finally for the high-producing tumour necrosis factor alpha (TNFα) -308 promoter A allele (rs180029, OR = 1.7, 95\% CI [1.2–2.4], \(p_c = 0.004\)). Although compound genotypes of alleles at these three polymorphisms were associated with disease risk, no formal evidence for epistatic interaction (evaluated with logistic regression) between the associated genotypes was detected.

Discussion/conclusion: Our study provides suggestive evidence for involvement of genetic variation in MDR3, FXR, and TNFα in the pathogenesis of collagenous colitis. Patients with this disease available for genetic studies are scarce and the size of the study population is the main limitation of our findings. However, because of the biological relevance of these results, our study proposes important clues to the pathogenesis of collagenous colitis. Therefore, replication in independent case-control panels is warranted.
Are plasma oxysterols potential bio-markers of radiation-induced organ damages?

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Plasma oxysterols such as 7α-hydroxycholesterol (7-OH), 27-hydroxycholesterol (27-OH) and 24S-hydroxycholesterol (24-OH) could be physiological markers of cholesterol metabolism. There are synthesized respectively by CYP7A1 (liver), CYP27A1 (lung, endothelial cells and circulating macrophages) and CYP46A1 (brain). The aims of this study are to determine if these plasma oxysterols could be markers of cholesterol metabolism disruption or tissue damage diagnostic, following irradiation of rats.

An HPLC technique has been developed, allowing the simultaneous assay of these three oxysterols in plasma. After alkaline hydrolyse of rat plasma samples (0.5 ml), extraction with hexane, and evaporation under nitrogen, the oxysterols were solubilised in HPbCD (HydroxyPropyl-b-CycloDextrin). They were then oxidized by the cholesterol oxidase and analysed by HPLC at 240 nm. Oxysterols identification has been performed by addition of internal standards (7-OH, 27-OH and 24-OH) in plasma samples.

The effects of a whole-body irradiation (8 Gy and 10 Gy) on plasma levels of the three oxysterols were studied 3 days after exposure in rat. The results indicate a significant decrease in 7-OH concentration after irradiation (-70% for both irradiation doses). A marked increase (x 5) in 24-OH plasma level was observed at 10 Gy whereas it was not significantly altered at 8 Gy. On the other hand, there is no change of the plasma level of 27-OH, whatever the irradiation dose.

This work demonstrated for the first time that oxysterols plasma levels were modified following irradiation. The changes of 7-OH plasma level suggest an alteration of the classical pathway of hepatic bile acids biosynthesis or a sign of liver damage. The variation of 24-OH plasma level could be due either to a modification of cholesterol metabolism in brain, or to changes in hepatic uptake of circulating 24-OH.
Reactive oxygen species are not involved in bile acid induced apoptosis of hepatocytes

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Background: Exposure of hepatocytes to hydrophobic bile acids induces apoptotic cell death, characterized by caspase activation and nuclear condensation. It has been hypothesized that bile acids generate reactive oxygen species (ROS) via activation of membrane-bound NADPH-oxidase and that these ROS activate the Src-family member Yes which in turn is responsible for bile acid induced cell death. This hypothesis suggests that anti-oxidant therapy might be useful in cholestatic disorders.

Aim: To investigate the role of ROS in bile acid induced death of hepatocytes.

Methods: Primary cultures of rat hepatocytes were exposed to the hydrophobic bile acid glycochenodeoxycholic acid (GCDCA: 50 μM) for 4 hours with or without the addition of anti-oxidants: pegylated catalase (PEG-CAT), pegylated superoxide dismutase (PEG-SOD), N-acetylcysteine (NAC) and the cell permeable glutathione-derivative GSH-monoethylester (GSH-MEE). Membrane-associated NADPH-oxidase was inhibited using diphenylene iodonium (DPI) and the Src-kinase family member Yes was inhibited using SU6656. Apoptosis was determined via caspase-3 activity assay and nuclear condensation and necrosis via Sytox green staining. The oxidative stress responsive gene heme-oxygenase-1 (HO-1) was used as a parameter of oxidative stress.

Results: None of the anti-oxidants tested abolished GCDCA-induced apoptosis, although PEG-SOD completely abolished superoxide anion (menadione) induced apoptosis and PEG-CAT completely abolished hydrogen peroxide induced necrosis. GCDCA did not induce HO-1 mRNA level, whereas menadione strongly induced HO-1 expression. The Yes inhibitor SU6656 inhibited GCDCA-induced apoptosis (-67%). Although the NADPH-oxidase inhibitor DPI reduced GCDCA-induced apoptosis (-75%), it strongly induced necrotic cell death (83% of cells).

Conclusion: Our results do not indicate any oxidative stress induced by bile acids. Furthermore, anti-oxidants do not reduce GCDCA-induced apoptosis. The proposed role of NADPH-oxidase in GCDCA-induced cell death is questioned, since inhibition of NADPH-oxidase does not protect against cell death, but rather shifts cell death from apoptosis to necrosis. Finally, a role of the Src-family member Yes in GCDCA-induced apoptosis is confirmed. Our findings suggest that bile acid induced cell death is not mediated via reactive oxygen species and that anti-oxidant therapy in cholestatic liver diseases is likely to be ineffective.
24-Nor-ursodeoxycholic acid as novel therapeutic strategy for inflammation-induced liver fibrosis in a murine model of
Schistosoma mansoni infection

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Introduction: The murine Schistosoma mansoni model is characterized by inflammation-induced, granulomatous liver fibrosis. Current antihelmintic therapy is ineffective once hepatic fibrosis is established. Thus there is an urgent need for an effective anti-fibrotic therapy for this type of liver fibrosis. Since we have shown previously that 24-nor-ursodeoxycholic acid (norUDCA) improves biliary fibrosis in a mouse model of sclerosing cholangitis (Mdr2-/-; Fickert et al 2006), we designed this study to test whether norUDCA is effective in non-cholestatic liver fibrosis.

Methods: NMRI mice were percutaneously infected with 50 S. mansoni cercariae, respectively. Liver damage, inflammation, hepatic fibrosis and bile duct injury were examined at different time points (8, 16, 24 weeks after infection). Therapeutic effects of bile acids (0.5% norUDCA-diet, 0.5% UDCA-diet) were studied after 4 weeks of treatment at 16 weeks after infection.

Results: Hepatic fibrosis was evident at all time points (8 weeks, 3-fold; 16 and 24 weeks 7-fold vs. uninfected control; p < 0.05), although serum parameters of liver damage remained unchanged. Interestingly, bile duct proliferation occurred only at 24 weeks after infection (K19: 3-fold, p < 0.05). NorUDCA but not UDCA reduced hepatic hydroxyproline content and alpha-SMA-expression 16 weeks after infection (2-fold and 1.5-fold respectively, norUDCA vs. untreated control, p < 0.05). Furthermore, norUDCA but not UDCA reduced hepatic fibrosis, granuloma size (33% of untreated controls, p < 0.05) and the amount of inflammatory cells.

Discussion/Conclusion: This study demonstrates (i) beneficial effects of norUDCA on granuloma size and hepatic fibrosis and (ii) anti-inflammatory properties of norUDCA and, therefore qualifying norUDCA as a promising drug for non-cholestatic liver fibrosis.
The role of bile salt toxicity in the pathogenesis of bile duct injury after non-heart-beating porcine liver transplantation

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Background: Intrahepatic bile duct strictures are a serious complication after non-heart-beating (NHB) liver transplantation. Bile salt toxicity has been identified as an important factor in the pathogenesis of bile duct injury and cholangiopathies. The role of bile salt toxicity in the development of biliary strictures after NHB liver transplantation is unclear.

Methods: In a porcine model of NHB liver transplantation, we studied the effect of different periods of warm ischemia in the donor on bile composition and subsequent bile duct injury after transplantation. After induction of cardiac arrest in the donor, liver procurement was delayed for 0 min (group A), 15 min (group B) or ≥ 30 min (group C). Livers were subsequently transplanted after four hours of cold preservation. In the recipients, bile flow was measured and bile samples were collected daily to determine the bile salt: phospholipid ratio. Severity of bile duct injury was semi-quantified by using a histological grading scale.

Results: Posttransplant survival was directly related to the duration of warm ischemia in the donor. The bile salt:phospholipid ratio in bile produced early after transplantation was significantly higher in group C, compared to group A and B. Histopathology showed the highest degree of bile duct injury in group C.

Conclusion: Prolonged warm ischemia in NHB donors is associated with the formation of toxic bile after transplantation, with a high biliary bile salt:phospholipid ratio. These data suggest that bile salt toxicity contributes to the pathogenesis of bile duct injury after NHB liver transplantation.
Polymorphisms of hepatobiliary phospholipid transporters
MDR-3 associated with non-anastomotic strictures after human liver transplantation

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Introduction: Non-anastomotic strictures (NAS) are considered to be the most troublesome biliary complication after liver transplantation. Although the pathogenesis of NAS is not completely clear, experimental studies have suggested that bile salt toxicity as a result of altered bile composition is involved. As hepatobiliary transporter proteins are responsible for bile secretion, we hypothesized that genetic variation in these proteins are associated with this risk of developing NAS.

Methods: Five hundred and twenty consecutive adult liver transplants were reviewed. Of these 520 procedures, cryopreserved splenocytes were available from 472 donors, and were used for genotyping. We studied genetic variation in the following genes: bile salt export pump (BSEP; transporter of bile salts), multi-drug resistant protein-3 (MDR-3; transporter of phospholipids) and multi-drug resistant related protein (MRP-2; transporter of glutathione and bilirubin). Four to five single nucleotide polymorphisms (SNP’s) with an equal physical distribution per gene were selected using a combination of Applera SNP browser and Hap Map data. Haplotypes were constructed using an Expectation-Maximization algorithm to estimate haplotype frequencies.

Results: NAS was detected in 77/472 patients (16%) after transplantation. Patients who received a donor liver with MDR3 haplotype ATCGT developed NAS almost twice as often (28%) as donor livers with other haplotypes (15%) (p = 0.007). ATCGT haplotype frequency among patients with NAS was 13.2% and 6.7% among patients without NAS. Analysis in a multivariate Cox regression model showed ATCGT haplotype of MDR-3 from the donor to be an independent risk factor for NAS (p = 0.004, OR = 2.23, 95% CI = 1.29–3.85). Haplotypes of BSEP and MRP were not associated with NAS.

Discussion/Conclusion: This study shows that a common haplotype in the transporter of phospholipids (MDR3) in donor livers is independently associated with a two-fold increased risk for NAS after OLT. We hypothesize that transport of phospholipids into the bile in patients who are carriers of this risk haplotype is diminished. This reduction of phospholipid transportation contributes to a more toxic bile composition, which is involved in the development of bile duct injury eventually leading to the clinical picture of NAS.
Phenotypic variability in BAAT-deficiency

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Introduction: BAAT (bile acid Coenzyme A: amino acid N-acyltransferase) conjugates bile acids with amino acid. Defects in BAAT have previously been reported in patients with mild or moderate disease, as has oligogenic inheritance, with mutation in BAAT and a 2nd gene.

Methods: Mutation screening: We PCR-amplified and sequenced coding sequence and splice junctions from genomic DNA. Mass spectrometry: Bile acid conjugation deficiency was detected by fast atom bombardment ionization mass spectrometry (FAB-MS) of patient urine and confirmed by GC-MS.

Results: A male infant of consanguineous parents developed severe cholestasis, necessitating liver transplantation. Genetic and biochemical studies demonstrated a deficiency in bile acid conjugation. The proband was homozygous for a premature stop codon in BAAT. Negative ion FAB-MS revealed an intense [M-H]^- ion at m/z 407 corresponding to unconjugated cholic acid, and accompanying ions at m/z 471 (sulphated dihydroxy-cholanoate) and m/z 467 and 483 (glucuronide conjugates of dihydroxy- and trihydroxy-cholanoates, respectively). Normal glycine- and taurine-conjugated primary bile acids were absent, consistent with defective bile acid amidation. Three of the proband’s 4 sisters were homozygous for the BAAT mutation, yet had mild phenotypes, largely only revealed upon biochemical testing. The proband may carry mutation in a 2nd gene, accounting for his more severe phenotype. We sequenced other ‘cholestasis genes,’ including ATP8B1, BSEP, JAG1, MDR3, and CLDN1, but did not detect a mutation.

Discussion/Conclusion: The proband may carry mutation in a 2nd, as-yet-unidentified gene. A whole-genome screen is underway to identify genomic regions in which such a mutation may lay.
Phenotypic severity in the Atp8b1-deficient mouse depends on genetic background

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Introduction: Atp8b1-deficient (mutant) mice serve as a model of FIC1/ATP8B1 disease. Studies have focused upon male mice in a 129 strain background; these mice manifest some features of human FIC1 disease, in mild form. We aimed to compare phenotypes of male and female wildtype (WT) and mutant mice in the C57Bl/6 (B6) and 129 strain backgrounds.

Methods: We evaluated 202 adult mice (125 mutant, 77 WT). After baseline serum collection, mice underwent weight-monitored short-term challenge with control or cholate-supplemented diet. Serum, bile, and liver tissue were collected at sacrifice; assays are underway. Preliminary phenotypic analyses identified significant findings, examples of which are discussed here.

Results:

BASELINE. Serum biochemistry: No differences in cholesterol or alkaline phosphatase (ALP) between WT and mutant mice were noted in the 129 background, but in the B6 background, mutant mice had lower cholesterol and higher ALP than WT. CHOLATE-SUPPLEMENTED DIET. Weight loss: Both strain and mutation status affected weight loss, with most rapid weight loss in mutant B6 mice. Serum bilirubin: Serum bilirubin was more highly elevated in mutant mice of B6, than in those of 129, background.

Discussion/Conclusion: Strain background modifies the Atp8b1-deficient phenotype. Atp8b1-deficient B6 mice may be a better model of human FIC1 disease than their 129 counterparts. Innate susceptibility to cholestasis also appears to differ between WT mice of these strains, indicating the importance of informed background strain selection for mouse models of liver disease. A study to identify genetic modifiers of the Atp8b1-deficiency phenotype is underway.
Differential effects of PFIC1 and BRIC1 mutations on protein stability and canalicular trafficking of ATP8B1

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Introduction: ATP8B1 mutations cause PFIC1, a progressive disease that without treatment leads to liver failure early in life, and the milder disorder BRIC1, in which patients suffer from episodic cholestatic attacks. In this study we examined how the genotypic variations relate to the phenotypic differences between both diseases.

Methods: We introduced three PFIC1 mutations (G308V, D554N, G1040R) and one BRIC1 mutation (I661T) by site-directed mutagenesis. Mutants were expressed in CHO-K1 cells and in the hepatocyte model cell line WIF-B9. mRNA and protein levels were analyzed using real-time PCR and Western blotting. The localization of ATP8B1 mutants was studied by confocal laser scanning microscopy and cell surface biotinylation. The interaction of ATP8B1 with its subunit/chaperone CDC50A was analyzed by co-immunoprecipitation.

Results: Protein levels of G308V, D554N, G1040R, and I661T were reduced to 11, 23, 64 and 33% of wild-type ATP8B1, respectively, while mRNA levels were not affected. Incubation with a proteasome inhibitor restored all mutant protein levels. In WIF-B9 cells, all PFIC1 mutants were retained in the endoplasmic reticulum, whereas the BRIC1 mutant was also detectable in the canalicular membrane. In CHO-K1 cells, only the I661T (BRIC1) and G1040R (PFIC1) mutants formed a complex with CDC50A and were detected at the plasma membrane.

Discussion/Conclusion: PFIC1 mutations resulted in loss of CDC50A interaction, impaired canalicular trafficking, and subsequent increased proteosomal degradation; however, although less stable, the G1040R mutant did interact with CDC50A and reached the plasma membrane. Similarly, the BRIC1 mutant protein was also less stable, but associated with CDC50A, and reached the canalicular membrane.
Increased coupling of biliary lipid to bile salt secretion in mice with genetic inactivation of bile salt export pump (Bsep)

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Introduction: Human BSEP gene mutations cause progressive familial intrahepatic cholestasis type 2. In Bsep-/- mice, biliary secretion of bile salts (BS) is decreased profoundly, but that of phospholipids (PL) and cholesterol (CH) is elevated (PNAS 2001; 98: 2011). We aimed to elucidate the discrepancy between low BS secretion and high biliary lipid secretion in Bsep-/- mice.

Methods: After bile collection for 90 min via gallbladder canulation, we infused tauro-beta-muricholate to Bsep-/- and Bsep+/+ (control) mice (C57J/Bl6 inbred mice) in stepwise increasing dosages (IV, 150–600 nmol/min; n = 9–12/group). Tauro-beta-muricholate was chosen since the secretion of beta-muricholate was relatively preserved in Bsep-/- mice. Biliary bile flow and biliary BS, PL and CH secretion rates were determined.

Results: Before tauro-beta-muricholate infusion, bile flow, BS and PL secretion were similar in Bsep+ and control mice, whereas biliary CH secretion was higher in Bsep-/- mice (+183%, p < 0.001). Tauro-beta-muricholate infusion increased bile flow, although significantly less in Bsep-/- (+20%) compared with controls (+79%; p < 0.05). Biliary PL-BS and CH-BS ratios were continuously 4–6 fold higher in Bsep-/- mice (p < 0.05). After infusion, biliary BS concentration (~90 mM) and composition (~90% beta-muricholate) were similar in Bsep+ and control mice. Hepatic mRNA expression of Mdr2, Abcg5 and Abcg8 were each 60–70% higher in Bsep-/- mice (each p < 0.05).

Discussion/Conclusion: Bsep-/- mice have increased coupling of biliary lipid (PL, CH) to BS secretion. The high biliary lipid secretion in Bsep+ mice cannot be attributed to changes in biliary bile flow, BS concentration or composition, but is rather due to increased expression of Mdr2, Abcg5 and Abcg8.
Gallstone disease in Swedish twins is linked to ABCG8 D19H risk genotype

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Introduction: Recently, the D19H variant of the hepatocanalicular cholesterol transporter ABCG8 was found to be strongly linked to gallstone disease (GD) in Caucasians. We now investigated this polymorphism in Swedish Twins.

Methods: The Swedish Twin Registry was merged with the Outpatient Discharge registry for gallstone related ICD diagnoses and screened for monozygotic (MZ) twins born between 1915 and 1956 with GD living in the Stockholm area. Abdominal ultrasound was performed in the MZ twins with undefined GD, and three concordant dizygotic (DZ) twin pairs were included; 88% of twins were females. ABCG8 D19H genotyping was performed using PCR-based assays with 5'-nuclease and fluorescence detection (TaqMan). For statistics, non-parametric linkage (NPL) score analysis was employed.

Results: Overall prevalence of D19H among affected twins was 30.4%. Of 25 MZ twin pairs, 20 were concordant and 5 were discordant for GD. Hetero- or homozygous 19H carriers were observed in 8 twin pairs; 7 of these were concordant for GD. With a 19H allele frequency of 10.3% in healthy Swedish controls, an NPL score of 3.95 (p < 0.0001) was estimated, indicating significant linkage of ABCG8 D19H to gallstones.

Discussion/Conclusion: These data from twins as perfectly matched affected pairs confirm the D19H variant of the cholesterol transporter ABCG8 as risk factor for GD.
Development of an in vitro model to study ABCB4 mutations implicated in intrahepatic cholestasis of pregnancy

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Introduction: Intrahepatic cholestasis of pregnancy (ICP) has a complex aetiology and several studies by ourselves and others implicate mutations in ABCB4 (e.g. A546D, R150K, S320F) in a subset of cases. However, the effects of these mutations on ABCB4 function and their response to oestrogens or progesterones have yet to be studied.

The aim of this work was to establish a high throughput in vitro system to investigate the effects of genetic variation in ABCB4 on the trafficking and function of the protein to elucidate the molecular mechanisms that underlie the ABCB4-related aetiology of cholestasis.

Methods: ABCB4 was transiently-expressed in HEK293T cells. Protein expression and localization were analyzed by Western-blot and confocal microscopy respectively. The functionality of ABCB4 was investigated using a fluorescent phosphatidylcholine derivative (NBD-PC) and by measuring the efflux of endogenous PC (using an enzyme-coupled colorimetric assay) and cellular cholesterol (enzyme coupled-fluorimetric assay).

Results: Western-analyses and confocal microscopy show the ability of these cells to express ABCB4 at the plasma membrane. Studies of NBD-PC or endogenous PC and cholesterol efflux from transiently transfected cells indicate the functionality of ABCB4 to export choline phospholipids to the culture medium in response to added bile acids.

Discussion/Conclusion: Comparison of the activity of wild-type and mutant ABCB4 suggest that this transient system can be used to explore the correlation between genotype, functionality of the protein, and patient phenotype.
Ursodeoxycholic acid treatment in preterm infants: A pilot study for the prevention of TPN-associated cholestasis

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Introduction: Intestinal fat digestion requires adequate lipase activity and bile acid concentrations, both of which are low in preterm infants.

Methods: A prospective, double-blind, placebo-controlled trial was conducted to evaluate the efficacy and safety of ursodeoxycholic acid (UDCA) use in preterm infants. Infants with gestational age ≤ 34 weeks and requiring total parenteral nutrition were randomly assigned to either UDCA or placebo treatment. The intervention started in the 3rd day of life with 5 mg/kg/day, increased to 10 mg/kg/day with the initiation of enteral feeding and continued with 20 mg/kg/day during full enteral feeding till 6th week of life. Only human milk was fed to the infants. The primary outcome measures were changes in fecal fat excretion and time to achieve full enteral feeding. Markers of cholestasis, growth, and nutritional-metabolic status were evaluated as secondary outcomes.

Results: Although fecal fat excretion slightly decreased, and achievement of full enteral feeding was earlier in the UDCA group, these differences were non-significant. Interestingly, while serum gamma-glutamyl transferase (γ-GT) activity increased during parenteral nutrition period in the placebo group, we observed a constant and significant decrease in UDCA group (102.7 ± 79.1, 72.4 ± 54.3, and 56.1 ± 36.0 U/l, at baseline, at 3–4th weeks and at 6th weeks, respectively).

Discussion/Conclusion: Given the fact that γ-GT is the early marker of TPN-associated cholestasis, this observation warrants further investigation to determine the utility of UDCA in preventing cholestasis for the infants destined to prolonged parenteral nutrition.
Treatment with ursodeoxycholic acid, but not with cholic acid, increases bile flow independently of cystic fibrosis transmembrane regulator in mice

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Introduction: Ursodeoxycholic acid (UDCA) treatment is frequently applied for cystic fibrosis-related liver disease (CFLD). The aim of our study was to determine if the choleretic activity of different bile salt (BS) treatments depends on the presence of Cystic fibrosis transmembrane conductance regulator (CFTR).

Methods: Bile flow (BF) was determined after gallbladder cannulation. The BF in the first 30 minutes was regarded as basal BF. Hereafter taurocholic acid (TCA) or tauroursodeoxycholic acid (TUDCA) were IV administered in stepwise increasing dosages, up to 1200 nmol/min/100g BW, to Cftr-null and WT mice on standard diet. Other Cftr-null mice and WT were fed either diet supplemented with cholic acid (CA) or UDCA, 0.5wt%, for 3 weeks before bile collection.

Results: The basal BF was similar in Cftr-null mice, compared to controls (6.3 ± 1.8 vs. 6.2 ± 0.1 µl/min/100 g BW, resp.; NS). IV administration of TCA or TUDCA to Cftr-null mice and WT increased BF to similar extents. Dietary CA treatment increased BF significantly less in Cftr-null mice than in controls (13.7 ± 3.0 vs. 17.9 ± 1.7 µl/min/100 g BW, resp.; p < 0.05). Dietary UDCA treatment increased BF more than dietary CA treatment but to similar levels in Cftr-null and WT mice (+ ~500%, 29.0 ± 5.9 vs. 31.0 ± 4.4 µl/min/100 g BW, resp.; NS).

Discussion/Conclusion: After acute administration, the capacity of bile salts to induce bile flow is not dependent of CFTR. After chronic BS treatment, however, only the BF induction by UDCA, but not by CA, is independent of CFTR. We hypothesize that the CFTR independent choleretic effect of UDCA is clinical important in the treatment of CFLD.
A single centre experience of dominant biliary strictures and cholangiocarcinoma in primary sclerosing cholangitis (PSC)

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Introduction: In patients with PSC, dominant biliary strictures can be managed endoscopically, but its impact on disease progression and the risk of cholangiocarcinoma (CC) development remains unclear.

Methods: We describe a 20 year experience of endoscopic therapy in 112 patients undergoing ERCP for PSC at a single centre.

Results: During an average follow-up of 4 (range, 0–19) years, 112 patients with PSC (69 M, 43 F; mean age 58 years) underwent a median of 2 (1–22) ERCPs. In the 65 patients with dominant biliary strictures, there was a median of 4 (0–32) interventions, compared to 0 (0–7) in the 47 without dominant strictures (p < 0.001). Endoscopic interventions included: (i) stenting alone (46%), (ii) dilatation alone (20%), (iii) dilatation and stenting (17%), and (iv) failed intervention (17%), (of which most required percutaneous drainage). There were no procedure-related deaths. The incidence of cholangiocarcinoma (CCA) during follow-up was higher in those with (13 of 65, 20%), compared to those without (0 of 47), dominant strictures (p = 0.001). Patients developed cholangiocarcinoma a mean of 5.9 years (0–21 years) after the diagnosis of PSC with a mean CA19.9 of 14,971 IU/L (9–84,000) compared to 74 IU/L in the benign group. Mean scores for disease models at diagnosis of cholangiocarcinoma were: Mayo PSC risk score 1.869, MELD 11.259 and UKELD 50.93 compared to 0.573, 7.45 and 45.5 respectively in the benign group. Mean time to death from diagnosis of cholangiocarcinoma was 8 (1–23) months.

Discussion/Conclusion: In patients with PSC and dominant strictures, repeated endoscopic therapy appears to be safe but in the present series there was a high risk of developing cholangiocarcinoma during long-term follow up.
Outcome of primary sclerosing cholangitis (PSC) in France: A four year prospective study of 150 patients treated with ursodeoxycholic acid (UDCA)

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Introduction: PSC is a rare disease and large-scale report of PSC in France was lacking. As a consequence, we initiated in 1997 a prospective multicenter observational study.

Methods: 150 patients with PSC (95 males, 55 females, median age: 43 [13–74] yrs) were included. Duration of PSC before inclusion was 2.9 [0–25] yrs. Ninety patients (60%) had associated inflammatory bowel disease. At entry, 12 patients (8%) had a diagnosis of malignancy (cholangiocarcinoma: n = 5, hepatocellular carcinoma: n = 2, gallbladder carcinoma: n = 2, colorectal cancer: n = 4). 140 patients (93%) were treated with UDCA (mean dosage: 13 mg/kg/d, started before inclusion in 117 patients (78%) or shortly after inclusion in 23 (13%). The median baseline biochemical characteristics were as follows: serum bilirubin: 12.5 μmol/l, alkaline phosphatase: 1.5 ULN, ALT: 1.2 ULN, serum albumin: 40 g/l. Median follow-up was 3.9 [0.1–7.2] yrs. The observed survival was compared to the predicted survival by the revised Mayo model.

Results: During follow-up, colorectal cancer was diagnosed in 4 patients and cholangiocarcinoma in one. Neither hepatocellular carcinoma nor gallbladder carcinoma occurred. Ten patients (6.7%) died and 26 (17%) were transplanted. Kaplan-Meier transplant-free survival at 4 years was 79% for the whole cohort and 82% for the 142 patients without history of hepatobiliary malignancy. Death was related to cancer (n = 5) (colorectal cancer: n = 3, cholangiocarcinoma: n = 2), liver failure (n = 4) or non-digestive cause (n = 1). Indications for liver transplantation were end-stage liver disease (n = 16), recurrent acute cholangitis (n = 4), biliary cancer (known or suspected) (n = 5) or intractable pruritus (n = 1). In multivariate analysis, predictive factors of death or transplantation were alkaline phosphatase > 3 ULN (p = 0.001), platelet count < 150,000/mm² (p = 0.003) and serum bilirubin > 22 μmol/l (p = 0.02). Observed survival and predicted survival were similar.

Discussion/Conclusion: PSC in France shares common features with other European and American series. Under low-dose UDCA, PSC remains a severe disease, the main cause of death or transplantation being liver failure. In keeping with other studies, the very low incidence of cholangiocarcinoma supports a potential chemoprotective effect of UDCA against development of biliary neoplasia.
Results of the French study of risk factors for primary biliary cirrhosis

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Introduction: Primary biliary cirrhosis (PBC) is a complex disease thought to result from combination of genetic and environmental factors. We report here the results of a large case-control study conducted in France aiming to investigate the familial, medical and lifestyle factors associated with PBC.

Methods: 222 patients with PBC (89% female; mean age, 51 years) and 509 unrelated controls matched for sex, age and geographical location were subjected to a standardized questionnaire regarding demographics, lifestyle, personal and familial medical history and reproductive history.

Results: Having a first-degree relative with PBC (Adjusted Odds Ratio 6.82; 95% confidence interval 1.16–52.93) or with autoimmune thyroid disease (AOR 5.30; 95% CI 1.38–28.07), personal history of urinary tract infections (UTI) (AOR 1.89, 95% CI 1.26–2.84), or past history of active or passive smoking (AOR 3.12, 95% CI 1.95–5.00) were significantly associated with increased risk of PBC. A history of use of hormone replacement therapy was not more frequent in women with PBC, but the past use of oral contraceptives was found to be associated with a decreased risk of the disease (AOR 0.64, 95% CI 0.43–0.95). The mean number of pregnancies was similar in the two groups, but the age at first pregnancy was significantly lower in PBC than in control women.

Discussion/Conclusion: The present data: (a) confirm the major genetic predisposition to autoimmune disorders and PBC; (b) reinforce the strong association between UTI, tobacco smoking and risk of PBC development; (c) suggest that exogenous estrogens may confer protection against PBC.
Ursodeoxycholic acid treatment for unconjugated hyperbilirubinemia: Promising results in Gunn rats

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Introduction: We aim to develop an oral treatment for unconjugated hyperbilirubinemia. Oral bile salt therapy is used extensively for cholestatic conditions with conjugated hyperbilirubinemia. It has remained unclear, however, whether bile salts could be useful as treatment for unconjugated hyperbilirubinemia. We determined the effects of dietary treatment with ursodeoxycholic acid (UDCA) or cholic acid (CA) in the Gunn rat model of unconjugated hyperbilirubinemia.

Methods: Gunn rats were fed standard diet or the same diet supplemented with UDCA (0.5 wt%) or CA (0.5 wt%) for either 1 wk or 6 wks. After 6 wks, 3H-UCB (0.3 Ci/100 g BW) was IV administered and UCB kinetics were determined over a 3 day period. UCB and 3H-UCB were measured in plasma, bile and feces during the experiments.

Results: UDCA or CA treatment for 1 wk decreased plasma UCB concentrations (-21% and -30%, resp.) compared to controls and was effective within 3 days. UDCA or CA treatment for 6 wks resulted in a stable decrease (~-40%, both groups) in plasma UCB concentrations from week 2 on. Both treatments transiently increased fecal output of UCB (+56% and +25%, resp.). 3H-UCB kinetic studies learned that both treatments decreased UCB pool size (~-33% and -32%, resp.) and increased fractional turnover.

Discussion/Conclusion: Dietary treatment with either UDCA or CA induces a sustained decrease in plasma UCB concentrations in Gunn rats. The mechanism involves stimulation of UCB turnover and fecal disposal. Present results support the feasibility of oral bile salt treatment in patients with unconjugated hyperbilirubinemia.
**Vitamin A deficiency dramatically aggravates obstructive cholestasis in rats, but is prevented by acute vitamin A therapy**

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**Introduction:** Cholestasis is commonly associated with reduced vitamin A levels. This may cause a disbalance in the ligands for the nuclear receptors farnesoid X receptor (FXR; activated by bile salts) and the retinoic X receptor-alpha (RXRα, activated by the vitamin A-derivative 9-cis-retinoic acid) which together regulate gene expression for bile salt synthesis and transport. Here, we studied the effect of vitamin A on liver damage inflicted by bile duct ligation (BDL) in rats.

**Methods:** Rats were fed a vitamin A-deficient (VAD) diet until serum retinol levels dropped to approximately 10% of control animals. VAD and control rats were exposed to BDL, with or without daily IP-injections with retinyl palmitate. Rats were sacrificed 7 days post-BDL and serum and liver samples were taken for mRNA, protein and histochemical analyses.

**Results:** VAD-BDL rats dramatically lost weight (-15% in 7 days), significantly more than sham- or BDL-treated control rats (-5%). Serum levels of aspartate transaminase (AST; 3161 ± 2358 U/L) and alanine transaminase (ALT; 630 ± 595 U/L) were strongly increased in VAD-BDL animals compared to control-BDL (259 ± 71 U/L and 82 ± 19 U/L, respectively) and sham-operated rats. Macro- and microscopic analysis of the VAD-BDL livers revealed numerous necrotic regions. VAD-BDL rats receiving retinyl palmitate were comparable to BDL rats fed a vitamin A sufficient diet.

**Discussion/Conclusion:** Vitamin A deficiency dramatically aggravates liver damage caused by obstructive cholestasis, but is efficiently treated through acute vitamin A supplementation. Biochemical analyses are currently underway to study the role of bile salts and FXR/RXRα target genes in this liver disease model.
An investigation into the chronic effects of deoxycholic acid in human colonic epithelial cells

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Introduction: We have previously shown that, in contrast to its well-documented prosecretory effects at pathophysiological (mM) concentrations, at more physiological (μM) levels, deoxycholic acid (DCA) is antisecretory. Here, we investigated possible mechanisms involved.

Methods: Cl⁻ secretion was measured as changes in short-circuit current (ISC) across voltage-clamped T₈₄ cell monolayers. Changes in intracellular Ca²⁺ were measured by fluorescence microscopy and protein phosphorylation/expression was measured by western blotting.

Results: DCA (100 μM for 24 hours) inhibited Cl⁻ secretory responses to the Ca²⁺- and cAMP- dependent agonists, carbachol and forskolin to 39.4 ± 7.7% (n = 10; p < 0.001) and 73.3 ± 5.9% (n = 5; p < 0.01) of those in control cells, respectively. This antisecretory effect was apparent within 3 hrs, and maximal after 6 hrs exposure to the bile acid. While DCA rapidly induced phosphorylation of the epidermal growth factor receptor (EGFr) and the ERK and p38 isoforms of MAPK, only EGFr inhibition reversed its antisecretory effects. Chronic exposure to DCA did not alter agonist-induced mobilisation of intracellular 2nd messengers nor did not alter the activity or expression of several key components of the Cl⁻ secretory pathway.

Discussion/Conclusion: The opposing pro- and antisecretory effects of DCA at different concentrations suggest a novel function for the bile acid as a colonic osmosensor. While our studies suggest transactivation of the EGFr is involved, the antisecretory actions of DCA do not appear to involve alterations in 2nd messenger production or in the activity or expression of key transport proteins. Ongoing studies aim to further elucidate mechanisms by which bile acids regulate colonic epithelial secretory function.
Influence of different sterol diets on hepatic and faecal cholesterol and bile acid concentrations in the guinea pig

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Introduction: The aim of the study was to investigate the influence of different cholesterol and/or sitosterol diets on the hepatic and faecal bile acid concentration in guinea pigs.

Methods: Four groups of female Dunkin Hartley guinea pigs were fed either a basal diet (“control”, n = 6), or a basal diet containing 0.05% cholesterol (“low-chol”, n = 8), 0.2% cholesterol (“high-chol”, n = 7), and 0.2% cholesterol and 0.5% unesterified sitosterol (“high-chol + sito”, n = 8) for a period of two weeks. Cholesterol, primary and secondary bile acids were analysed in liver tissue and faeces.

Results: Hepatic cholesterol concentration was significantly increased in the animals fed the high-cholesterol diet compared to the animals of the other groups (control vs. low-chol vs. high-chol vs. high-chol + sito: 6.16 ± 1.34 vs. 6.48 ± 0.96 vs. 14.6 ± 2.1 vs. 7.57 ± 0.94 mg/g dry matter). These hepatic cholesterol concentrations were confirmed by the faecal total bile acid concentrations. Thus, the significantly highest total bile acid concentration was found in the faeces of the animals fed the high-cholesterol diet (control vs. low-chol vs. high-chol vs. high-chol + sito: 117 ± 27 vs. 246 ± 70 vs. 380 ± 138 vs. 256 ± 89 μg/g dry matter), although the hepatic total bile acid concentration was diminished in all sterol supplemented groups compared to the control group.

Discussion/Conclusion: The feeding of high cholesterol doses increases the body cholesterol pool of guinea pigs and results in elevated cholesterol elimination also due to the bile acid excretion.
Effect of treatment with rifampicin and ursodeoxycholic acid on circulating fibroblast growth factor 19 and bile acids in man

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Introduction: Fibroblast growth factor 19 (FGF-19) is involved in the regulation of bile acid biosynthesis and gallbladder filling. Its expression is regulated by the farnesoid X receptor (FXR) and is suppressed by oral chenodeoxycholic acid (CDCA). We now aimed to study the effects of rifampicin (RIFA), ursodeoxycholic acid (UDCA) or a combination of both treatments on circulating FGF-19 levels.

Methods: Thirty patients scheduled for laparoscopic cholecystectomy were randomized to RIFA (600 mg/day during one week before surgery), UDCA (one g/day during three weeks before surgery) or a combination of both treatments. Twenty untreated patients served as controls. Fasting serum levels of FGF-19 and 7α-hydroxy-4-cholesten-3-one (C4; a marker of bile acid synthesis) were analyzed by ELISA and HPLC, respectively, prior to and after treatment.

Results: Treatment with RIFA or UDCA alone or in combination did not alter circulating FGF-19 levels (before/after treatment; RIFA, 150 ± 44 and 127 ± 44 pg/ml; UDCA, 113 ± 70 and 94 ± 49 pg/ml; RIFA + UDCA, 147 ± 53 and 131 ± 71 pg/ml; controls, 137 ± 84 pg/ml). However, RIFA and RIFA + UDCA significantly (paired analysis) increased C4, in RIFA by 48.2% (p = 0.023) and in RIFA + UDCA by 58.5% (p = 0.012) groups. C4 was unchanged by UDCA.

Discussion/Conclusion: Fasting serum levels of FGF-19 show a wide scatter that are not changed by oral UDCA, in contrast to CDCA, in accordance to the weaker binding to nuclear receptor FXR. Stimulation of bile acid synthesis by RIFA due to binding to nuclear receptor PXR, a possible detoxification mechanism, is sustained by combining RIFA with UDCA.
The bile acid UDCA enhances the anti-metastatic and anti-tumor action of CPT-11

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Introduction: Camptothecin (CPT-11/SN-38) presents anti-tumor activity through inhibition of the DNA topoisomerase I and is used as a second line therapeutic agent in advanced colorectal cancers. Ursodeoxycholic acids (UDCA) facilitates apoptosis of colorectal adenocarcinoma cells treated with SN-38. This study examined the combine effects of CPT-11 and UDCA on the inhibition of hepatic metastasis and proliferation of colon cancer (MC26) cells, both in vivo and in vitro.

Methods: Transplantable GFP-transfected MC26 cells were injected into the spleen capsule of BALb/c mice and the liver tumors were studied. The mice received CPT-11 injection IP every 2-3 days and had access to normal or UDCA-containing diets for 3 weeks. Cultured MC26 cells were treated with CPT-11/SN-38 lactone or carboxylate, and further incubated with and without UDCA for 48 hours.

Results: The incidences of hepatic metastasis were decreased by 25% in both the CPT-11 and UDCA groups and by 30% in the combined CPT-11/UDCA group. The liver tumor size evaluated by tissue weight and GFP intensity was significantly decreased in the CPT-11, UDCA and CPT-11/UDCA groups when compared to control. The proliferation and migration of cultured MC26 cells analyzed by the MTT and wound-healing assays, respectively, were significantly decreased in the CPT-11/SN38 treated cells compared to control, and the cell proliferation was significantly intensified in the presence of UDCA.

Discussion/Conclusion: Camptothecin and UDCA decrease hepatic metastasis and proliferation of colon cancer cells, and the combined administration enhances these effects. Therefore, the combined administration of both agents could have clinical relevance against colorectal cancer metastasis.
Nasobiliary drainage induces complete and long-lasting remission in benign recurrent intrahepatic cholestasis; an update

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Introduction: FIC1 disease, caused by mutations in ATP8B1, is a continuum between benign recurrent intrahepatic cholestasis (BRIC1) and progressive familial intrahepatic cholestasis (PFIC1). Patients with BRIC present with attacks of cholestasis; the resulting pruritus is severely disabling and long lasting cholestasis may induce liver fibrosis. We recently started using temporary interruption of the enterohepatic circulation by nasobiliary drainage (NBD) to abort cholestatic episodes in BRIC patients. In 2006 we published the promising results of 4 effective NBD procedures in 3 BRIC1 patients. Here, we provide an up-date of our experience.

Methods: So far, 12 cholestatic attacks in 5 BRIC1 patients were treated by NBD in the UMC Utrecht. Bile diversion was established by endoscopically introducing a multihole nasobiliary drain into the ductus choledochus during a cholestatic episode, which was removed after 1 week.

Results: In 8/12 treatments, pruritus totally disappeared within 48 hours and serum bile acid levels returned to normal or near normal levels. In 4/12 treatments, NBD could not ameliorate the cholestatic episode. Retrospectively, in 2 patients (n = 3; one patient had two attempts at bile drainage by NBD) this was attributed to extensive liver fibrosis present at the time of NBD. Both patients are currently doing well with partial biliary drainage (PBD).

Discussion/Conclusion: NBD constitutes an effective therapy for patients with BRIC1 without progression of liver disease. It greatly improves quality of life, and, when instituted early, it may prevent or ameliorate progression of liver disease. PBD can be considered when NBD fails to resolve a cholestatic episode.
Protection of oesophageal cells from DCA mediated apoptosis by UDCA treatment

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Introduction: Gastro-oesophageal reflux disease (GORD) and its sequela, Barrett’s oesophagus (BO) are major risk factors for the development of oesophageal adenocarcinoma (OAC). One of the main constituents of the refluxate in GORD and BO are bile acids such as deoxycholate (DCA). Hydrophobic bile acids are known to be toxic at high doses causing apoptosis which may be attenuated by treatment with hydrophilic bile acids such as UDCA. The aim of this study is to investigate the apoptotic effect of DCA in oesophageal cell lines and any potential protective effect exerted by pre-treatment with UDCA.

Methods: Apoptosis was measured by Annexin-V and Propidium-iodide staining to differentiate between early and late apoptosis by flow cytometry. UDCA pre-treatment was tested under two strategies:– Pre-treatment followed by co-incubation with apoptotic concentrations of DCA (500 uM) or pre-treatment followed by removal of UDCA prior to DCA exposure.

Results: DCA induced apoptosis both in a time (max-6 Hrs) and dose-dependant manner in the cell lines tested (HET-1A-and-SKG4). UDCA had no apoptotic effect under same conditions. Excitingly, UDCA pre-treatment of oesophageal cells (SKGT4) protected them from DCA mediated apoptosis (27.41% increased-survival) even when UDCA was removed prior to DCA exposure (25.74% increased-survival).

Discussion/Conclusion: This study demonstrated a novel protective role of UDCA in oesophageal cells. This effect was pharmalogical in fashion as the protective effect continued upon removal of UDCA prior to DCA stimulation. The development of UDCA analogs and further analysis of UDCA in an oesophageal setting may yield novel strategies towards therapies for GORD and BO.
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